



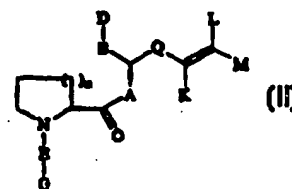
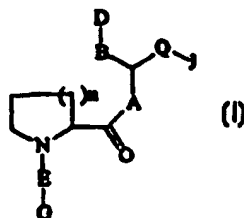
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|--|--|
| (51) International Patent Classification ⁶ : C07D 211/60, 401/12, 409/12, 405/06, 405/14, 405/12, A61K 31/445 | | A1 | (11) International Publication Number: WO 97/36869 |
| | | | (43) International Publication Date: 9 October 1997 (09.10.97) |
| (21) International Application Number: PCT/US97/04916 | | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). | |
| (22) International Filing Date: 24 March 1997 (24.03.97) | | | |
| (30) Priority Data: 08/626,259 29 March 1996 (29.03.96) US | | | |
| (71) Applicant: VERTEX PHARMACEUTICALS INCORPORATED [US/US]; 130 Waverly Street, Cambridge, MA 02139-4242 (US). | | | |
| (72) Inventors: ARMISTEAD, David, M.; Five Cutting Drive, Maynard, MA 01574 (US). SAUNDERS, Jeffrey, O.; 164 Parker Street, Acton, MA 01720 (US). | | Published With international search report. | |
| (74) Agents: HALEY, James, F., Jr. et al.; Fish & Neave, 1251 Avenue of the Americas, New York, NY 10020-1104 (US). | | | |

(54) Title: N-(2 OXOACETYL OR SULPHONYL)-PYRROLIDINE/PIPERIDINE-2-CARBOXYLIC ACID DERIVATIVES WITH IMPROVED MULTI-DRUG RESISTANCE ACTIVITY

(57) Abstract

The present invention relates to compounds of formula (I) and (II) that can maintain, increase, or restore sensitivity of cells to therapeutic or prophylactic agents. This invention also relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well-suited for treatment of multi-drug resistant cells, for prevention of the development of multi-drug resistance, and for use in multi-drug resistant cancer therapy.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|--|----|--|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav Republic of Macedonia | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | ML | Mali | TR | Turkey |
| BG | Bulgaria | HU | Hungary | MN | Mongolia | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MR | Mauritania | UA | Ukraine |
| BR | Brazil | IL | Israel | MW | Malawi | UG | Uganda |
| BY | Belarus | IS | Iceland | MX | Mexico | US | United States of America |
| CA | Canada | IT | Italy | NE | Niger | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NL | Netherlands | VN | Viet Nam |
| CG | Congo | KE | Kenya | NO | Norway | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NZ | New Zealand | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's Republic of Korea | PL | Poland | | |
| CM | Cameroon | KR | Republic of Korea | PT | Portugal | | |
| CN | China | KZ | Kazakhstan | RO | Romania | | |
| CU | Cuba | LC | Saint Lucia | RU | Russian Federation | | |
| CZ | Czech Republic | LI | Liechtenstein | SD | Sudan | | |
| DE | Germany | LK | Sri Lanka | SE | Sweden | | |
| DK | Denmark | LR | Liberia | SG | Singapore | | |
| EE | Estonia | | | | | | |

N-(2 OXOACETYL OR SULPHONYL)-PYRROLIDINE/PIPERIDINE-2-CARBOXYLIC ACID
DERIVATIVES WITH IMPROVED MULTI-DRUG RESISTANCE ACTIVITY

TECHNICAL FIELD OF THE INVENTION

The present invention relates to novel compounds which can maintain, increase, or restore sensitivity of cells to therapeutic or prophylactic agents. This invention also
5 relates to pharmaceutical compositions comprising these compounds. The compounds and pharmaceutical compositions of this invention are particularly well-suited for treatment of multi-drug resistant cells, for prevention of the development of multi-drug resistance and for use in multi-drug resistant
10 cancer therapy. The present invention also relates to methods and pharmaceutical compositions for stimulating the growth of neurites in nerve cells.

BACKGROUND OF THE INVENTION

A major problem affecting the efficacy of
15 chemotherapy regimens is the evolution of cells which, upon exposure to a chemotherapeutic drug, become resistant to a multitude of structurally unrelated drugs and therapeutic agents. The appearance of such multi-drug resistance often occurs in the presence of overexpression of a 170-KD membrane
20 P-glycoprotein (gp-170 or MDR1). The gp-170 protein is present in the plasma membranes of some healthy tissues, in addition to cancer cell lines, and is homologous to bacterial transport proteins (Hait et al., Cancer Communications, Vol. 1(1), 35 (1989); West, TIBS, Vol. 15, 42 (1990)). The protein
25 acts as an export pump, conferring drug resistance through active extrusion of toxic chemicals. Although the mechanism for the pump is unknown, it is speculated that the gp-170 protein functions by expelling substances that share certain chemical or physical characteristics, such as hydrophobicity,

-2-

the presence of carbonyl groups, or the existence of a glutathione conjugate (see West).

Recently, another protein responsible for multidrug resistance, MRP (multidrug resistance associated protein), was identified in H69AR cells, an MDR cell line that lacks detectable P-glycoprotein [S. P. C. Cole et al., Science, 258, pp. 1650-54 (1992)]. MRP has also been detected in other non-P-glycoprotein MDR cell lines, such as HL60/ADR and MCF-7 breast carcinoma cells [(E. Schneider et al., Cancer Res., 54, pp. 152-58 (1994); and N. Krishnamachary et al., Cancer Res., 53, pp. 3658-61 (1993)].

The MRP gene encodes a 190 KD membrane-associated protein that is another member of the ATP binding cassette superfamily. MRP appears to function in the same manner as P-glycoprotein, acting as a pump for removing natural product drugs from the cell. A possible physiological function for MRP may be ATP-dependent transport of glutathione S-conjugates [G. Jedlitschky et al., Cancer Res., 54, pp. 4833-36 (1994); I. Leier et al., J. Biol. Chem., 269, pp. 27807-10 (1994); and Muller et al., Proc. Natl. Acad. Sci. USA, 91, pp. 13033-37 (1994)].

The role of MRP in clinical drug resistance remains to be clearly defined, but it appears likely that MRP may be another protein responsible for a broad resistance to anti-cancer drugs.

Various chemical agents have been administered to repress multi-drug resistance and restore drug sensitivity. While some drugs have improved the responsiveness of multi-drug resistant ("MDR") cells to chemotherapeutic agents, they have often been accompanied by undesirable clinical side effects (see Hait et al.). For example, although cyclosporin A ("CsA"), a widely accepted immunosuppressant, can sensitize certain carcinoma cells to chemotherapeutic agents (Slater et al., Br. J. Cancer, Vol. 54, 235 (1986)), the

-3-

concentrations needed to achieve that effect produce significant immunosuppression in patients whose immune systems are already compromised by chemotherapy (see Hait et al.). In addition, CsA usage is often accompanied by adverse side effects including nephrotoxicity, hepatotoxicity and central nervous system disorders. Similarly, calcium transport blockers and calmodulin inhibitors both sensitize MDR cells, but each produces undesirable physiological effects (see Hait et al.; Twentyman et al., Br. J. Cancer, Vol. 56, 55 (1987)).

Recently, agents have been developed which may be of potentially greater clinical value in the sensitization of MDR cells. These agents include analogs of CsA which do not exert an immunosuppressive effect, such as 11-methyl-leucine cyclosporin (11-met-leu CsA) (see Hait et al.; Twentyman et al.), or agents that may be effective at low doses, such as the immunosuppressant FK-506 (Epand and Epand, Anti-Cancer Drug Design 6, 189 (1991)). PCT publication WO 94/07858 refers to a novel class of MDR modifying agents with some structural similarities to the immunophilins FK-506 and rapamycin. Despite these developments, there is still a need for more effective agents which may be used to resensitize MDR cells to therapeutic or prophylactic agents or to prevent the development of multi-drug resistance.

Interestingly, compounds such as the immunophilin FK506 have been shown to not only be effective against multi-drug resistance, but also to be effective in stimulating neurite outgrowth. In co-pending United States patent application 08/486,004, a co-applicant of the present invention discovered that other MDR reversing compounds could also stimulate neurite outgrowth in the presence or absence of exogenous or endogenous NGF. These compounds all had the ability to bind the cellular protein FKBP12. FKBP12 appears to be linked to neurite outgrowth because expression of FKBP12

-4-

alone has been found to stimulate neurite outgrowth in nerve cells.

W. E. Lyons et al. [Proc. Natl. Acad. Sci. USA, 91, pp. 3191-95 (1994)] demonstrated that FK506 acts synergistically with nerve growth factor (NGF) in stimulating neurite outgrowth in a rat pheochromocytoma cell line. Interestingly, another immunophilin, rapamycin, did not inhibit the effects of FK-506 on neurite outgrowth, but rather was neurotrophic itself, displaying an additive effect with FK-506. In sensory ganglia, FK-506 demonstrated similar neurotrophic effects, but those effects were blocked by rapamycin. Both rapamycin and FK506 have the ability to bind to a cellular protein, FK506 binding protein or FKBP12.

These results led the authors to speculate that FK-506 was exerting its neurotrophic effect through its complexing with FKBP12 and calcineurin and inhibition of the latter's phosphatase activity. Alternatively, the authors proposed FK-506 was acting via a "stripping" mechanism, such as that involved in the removal of FKBP12 from membrane receptors, RyR and IP₃R.

In view of the wide variety of disorders that may be treated by stimulating neurite outgrowth and the relatively few FKBP12-binding compounds that are known to possess this property, there remains a great need for additional neurotrophic, FKBP12-binding compounds.

SUMMARY OF THE INVENTION

The present invention provides novel compounds that have a surprisingly improved ability, as compared with previously described MDR modifiers, to maintain, increase or restore drug sensitivity in multi-drug resistant ("MDR") cells, compositions containing these compounds and methods for

-5-

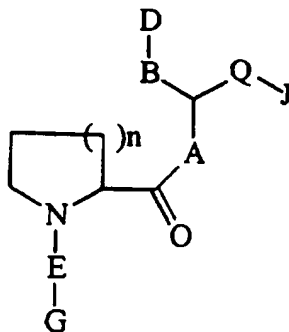
using them. The compounds of this invention may be used alone or in combination with other therapeutic or prophylactic agents to maintain, increase or restore the therapeutic or prophylactic effects of drugs in cells, especially MDR cells, or to prevent the development of MDR cells. According to one embodiment of this invention, these novel compounds, compositions and methods are advantageously used to aid or enhance chemotherapy regimens for the treatment or prophylaxis of cancer and other diseases. The present invention also provides methods for preparing the compounds of this invention and intermediates useful in those methods.

In another embodiment, the present invention also relates to methods and pharmaceutical compositions for stimulating the growth of neurites in nerve cells. The compounds of this invention bind FKBP12 and cause a significant increase in neurite outgrowth.

The neurotrophic compositions provided comprise a compound of this invention alone or together with a known neurotrophic factor. The methods described herein employ those compositions to effectuate neurite outgrowth and are thus useful to treat nerve damage caused by various diseases and physical traumas.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to two related classes of



-6-

compounds which have the ability either to increase toxicity of drugs in multi-drug resistant (MDR) cells and to stimulate neurite outgrowth in nerve cells. One of these classes of compounds is represented by formula (I):

5 (I)

wherein:

A is CH₂, O, NH or N-[(C1-C4)-alkyl];

10 B is (C1-C6)-straight or branched alkyl, or (C2-C6)-straight or branched alkenyl or alkynyl, wherein one of the carbon atoms of B is optionally replaced by O, S, SO, SO₂, NH or N-[(C1-C4)-alkyl];

15 D is 1-[(C1-C4)-alkyl]-4-piperidinyl; 1-piperazinyl; 1-[(C1-C4)-alkyl]-4-piperazinyl; a 5-7-membered cycloalkyl or cycloalkenyl ring optionally comprising substituents at the 3 and/or 4 position of said ring, wherein said substituents are selected from oxo, OH, (C1-C4)-alkyl, O-[(C1-C4)-alkyl], O-[(C2-C4)-alkenyl], NH₂, N,N di-[(C1-C4)-alkyl] amino or halogen; or a monocyclic or bicyclic aromatic ring structure consisting of 5 to 6 members in each ring and optionally
20 comprising up to 4 heteroatoms independently selected from N, O or S;

E is SO₂ or -C(O)-C(O)-;

25 G is 1-[(C1-C4)-alkyl]-4-piperidinyl, 1-piperazinyl, 1-[(C1-C4)-alkyl]-4-piperazinyl, (C1-C7)-straight or branched alkyl, (C2-C7)-straight or branched alkenyl or alkynyl, (C5-C7)-cycloalkyl, or a monocyclic or bicyclic aromatic ring structure consisting of 5 to 6 members in each ring; wherein up to two carbon atoms in any G are optionally replaced independently by O, S, SO, SO₂ or N;

30 wherein G optionally comprises up to three substituents independently selected from halogen, hydroxyl, (C1-C6)-straight or branched alkyl, (C2-C6)-straight or branched alkenyl, O-[(C1-C5)-straight or branched alkyl], O-[(C2-C5)-straight or branched alkenyl], O-benzyl, amino, carboxyl, N-

-7-

[(C1-C5)-straight or branched alkyl], N-[(C2-C5)-straight or branched alkenyl], trifluoromethyl or trifluoromethoxy; and wherein one carbon atom of any individual substituent is optionally replaced by O, N or S;

5 Q is a five membered aromatic ring containing 1 to 2 heteroatoms selected from N, O or S, or a six membered aromatic ring containing 0 to 2 heteroatoms selected from N, O or S;

J is a monocyclic or bicyclic aromatic ring structure
10 attached to the 3 position of Q consisting of 5 to 6 members in each ring, optionally comprising up to four heteroatoms independently selected from O, S, or N; and

wherein J optionally comprises up to 3 substituents independently selected from halo, OH, CH₂OH, NO₂, SO₃H,
15 trifluoromethyl, trifluoromethoxy, O-phenyl, 1,2-methylenedioxy, NR¹R², amino, carboxyl, N-[(C1-C5)-straight or branched alkyl]-carboxamide, N-[(C2-C5)-straight or branched alkenyl]-carboxamide, N-morpholinocarboxamide, N-benzylcarboxamide, N-
20 thiomorpholinocarboxamide, N-picolinoylcarboxamide, morpholinyl, piperidinyl, O-R³, CH₂-(CH₂)_q-R³, O-(CH₂)_q-R³, (CH₂)_q-O-R³, CH=CH-R³, (C1-C6)-straight or branched alkyl, or (C2-C6)-straight or branched alkenyl, wherein in any substituent one carbon atom is optionally replaced by a
25 heteroatom selected from the group consisting of O, S, SO, SO₂, NH or N-[(C1-C4)-alkyl];

wherein R¹ and R² are independently selected from the group consisting of hydrogen, (C1-C6)-straight or branched alkyl, (C2-C6)-straight or branched alkenyl or alkynyl and
30 benzyl;

R³ is selected from the group consisting of 4-methoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrazyl, quinolyl, 3,5-dimethylisoxazolyl, 2-methylthiazoyl, thiazoyl, 2-thienyl, 3-thienyl and pyrimidyl; and

-8-

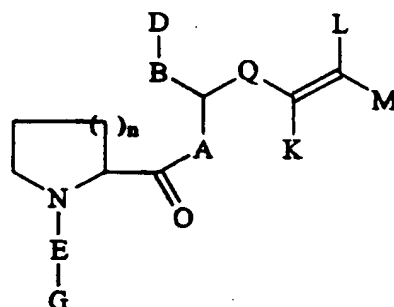
q is 0-2; and

n is 1 or 2.

The "3 position of Q" recited above is relative to the point of attachment of Q to the rest of the compound. For the purposes of this application, this point of attachment is designated the 1 position, regardless of any potential conflict with accepted chemical nomenclature.

The term "carbon atom", as used herein in reference to something that is "optionally replaced by" O, S, SO, SO₂, NH or N-[(C1-C4)-alkyl], includes C, CH, CH₂ or CH₃, depending upon where in an alkyl, alkenyl, alkynyl, cycloalkyl or cycloalkenyl chain or ring that carbon atom is located. Similarly, the reference to replacement by O, S, SO, SO₂, NH or N-[(C1-C4)-alkyl] includes O, OH, S, SH, SH₂, SO, SOH, N, NH, NH₂, N-[(C1-C4)-alkyl] and N(H)-[(C1-C4)-alkyl], again depending upon the location of the replacement in the chain or ring.

The other class of compounds of this invention are represented by formula (II):



(II)

wherein:

A, B, D, E, G and Q are as defined as above;

K is H, (C5-C7) cycloalkyl, (C5-C6) aromatic ring, 1-[(C1-C4)-alkyl]-4-piperidinyl, 1-piperazinyl, 1-[(C1-C4)-alkyl]-4-piperazinyl, (C1-C7)-straight or branched alkyl, (C2-C7)-straight or branched alkenyl or alkynyl, wherein up to two

-9-

carbon atoms in K are optionally replaced independently by O, S, SO, SO₂, NH, NO or N-(C1-C4)-alkyl,

wherein K optionally comprises up to 2 substituents independently selected from halo, amino, hydroxy, carboxy, methoxy or (C1-C3)alkyl; and

L and M are independently selected from H, (C1-C7)-straight or branched alkyl, (C2-C7)-straight or branched alkenyl or alkynyl, wherein one carbon atom in R² and R³ is optionally replaced by O, S, SO, SO₂, NH or N-(C1-C4)-alkyl,

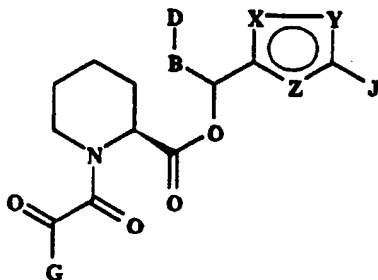
wherein L and M optionally comprise up to two substituents independently selected from halogen, hydroxy, amino, carboxy, or a 5 to 6 membered aromatic ring, said aromatic ring comprising up to two heteroatoms selected from N, O or S; and n is 1 or 2.

Compounds of formulae (I) and (II) of this invention include all optical and racemic isomers. The stereochemistry at positions 1 and 2 may be either R or S. In a preferred embodiment, the stereochemistry at position 1 is S.

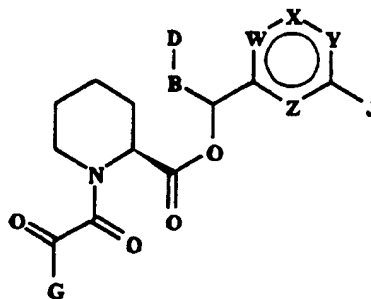
Preferably, none of the monocyclic or bicyclic rings that may be present in either compounds of formulae (I) or (II) contain more than one heteroatom per ring.

More preferably, in compounds of formulae (I) and (II), A is oxygen and E is -C(O)-C(O)-. Even more preferred are compounds wherein n is 2 and the potential location of heteroatoms in Q exclude the 1 and 3 positions (i.e., the position where the aromatic ring is bound to the rest of the molecule and the position where J is bound to the aromatic ring). These compounds are represented by formulae (III) and (IV):

-10-



(III)



(IV)

Preferably in compounds of formulae (III) and (IV):

B is propyl, ethyl, or 1-methylethenyl;

D is phenyl, N-morpholinyl, 4-hydroxy-cyclohexyl, 4-(N-methyl)-piperidinyl, 4-pyridyl or pyranyl;

G is 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl, 4-fluorophenyl, 2-furanyl, 1,1-dimethyl-2-methoxyethyl, t-butyl, 4-(4-hydroxy) pyranyl, isobutyl, 4-pyranyl, isobutyl, isopropyl, 1-methylcyclohexyl, 1,1,2-trimethylpropyl, 1-hydroxycyclohexyl, 1-trimethylpropyl, 4-methoxy-1-hydroxy-cyclohexyl, 5-methoxymethyl-2-methylphenyl, 2-methylcyclohexyl, 5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl-2-enyl, 2-methylcyclohexyl, 5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl, 5-ethoxy-2-methylcyclohexyl, 4-ethoxy-N-aceto-2-pyrrolidinyl, or 5-isopropyl-2-methylcyclohexyl; and

J is 4-phenyl-1-(3-pyridyl)-1-butenyl, 2,5-diethoxyphenyl, 4-phenyl-1-(3-pyridyl-N-oxide)-1-butenyl, 2-methoxyphenyl, 1-(3-pyridyl)-1-pentenyl, 2-ethoxyphenyl, 2,5-dipropoxyphenyl, 2,6-dimethoxyphenyl, 1-(3-pyridyl)-1-butenyl, 1-(3-pyridyl)-1-pentenyl, 1-(3-pyridyl)-1-hexenyl, 1-(4-methylphenyl)-1-pentenyl, 2,6-dimethoxymethylphenyl, 1-cyclohexyl-1-pentenyl, 2-ethoxymethyl-N-indolyl, 1-cyclohexyl-3-methoxy-1-propenyl, 2,6-diethoxymethylphenyl, 1-(3-pyridyl)-1-hexa-1,5-dienyl, 1-(4-pyranyl)-1-hexa-1,5-dienyl, 1-

-11-

cyclohexyl-1-hexenyl, 2,5-dipropyl-N-pyrrolyl, 2-methyl-5-butyl-N-pyrrolyl, 3-(1-methoxy)-2-hexenyl, 3-(1-methoxy)-4-methyl-2-pentenyl, 2,5-dimethyl-N-pyrrolyl, 3-(2-methyl)-3-heptenyl or 2-(2-hexenyl); and

- 5 W, X, Y and Z are independently selected from CH, N, O or S.

The most preferred compounds of this invention are represented by formulae (III) and (IV) with the other components and the orientation ("R/S") listed in the table below. Compounds of formula (III) are distinguished from those of formula (IV) by the lack of a "W" component (indicated by a "-" in the table).

15

| # | B | D | G | J | W | X | Y | Z | R/S |
|---|--------|--------|------------------------|------------------------------------|----|----|----|----|-----|
| 3 | propyl | phenyl | 3,4,5-trimethoxyphenyl | Z-4-phenyl-1-(3-pyridyl)-1-butenyl | CH | CH | CH | CH | R/S |
| 4 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-diethoxyphenyl | CH | CH | CH | CH | R/S |
| 5 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-4-phenyl-1-(3-pyridyl)-1-butenyl | CH | CH | CH | CH | S |
| 6 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-4-phenyl-1-(3-pyridyl)-1-butenyl | CH | CH | CH | CH | R |
| 7 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-diethoxyphenyl | CH | CH | CH | CH | S |

-12-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|--------|--------|------------------------|--|----|----|----|----|-----|
| 8 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-diethoxyphenyl | CH | CH | CH | CH | R |
| 9 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-4-phenyl-1-(3-pyridyl-N-oxide)-1-butenyl | CH | CH | CH | CH | R |
| 10 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-diethoxyphenyl | CH | N | CH | CH | R |
| 11 | propyl | phenyl | 3,4-dimethoxyphenyl | 2,5-diethoxyphenyl | CH | N | CH | CH | R |
| 5 12 | propyl | phenyl | 3,4-dimethoxyphenyl | 2,5-diethoxyphenyl | CH | N | CH | CH | S |
| 13 | propyl | phenyl | 3,4-dimethoxyphenyl | 2-methoxyphenyl | CH | N | CH | CH | R |
| 14 | propyl | phenyl | 3,4-dimethoxyphenyl | 2-methoxyphenyl | CH | N | CH | CH | S |

-13-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|--------|--------|------------------------|----------------------------|----|----|----|----|-----|
| 15 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2-methoxyphenyl | CH | N | CH | CH | R |
| 16 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-pentenyl | CH | CH | CH | CH | S |
| 17 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-pentenyl | CH | CH | CH | CH | R |
| 18 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2-ethoxyphenyl | CH | N | CH | CH | R |
| 5 19 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2-ethoxyphenyl | CH | N | CH | CH | S |
| 20 | propyl | phenyl | 3,4-dimethoxyphenyl | 2-ethoxyphenyl | CH | N | CH | CH | R |
| 21 | propyl | phenyl | 3,4-dimethoxyphenyl | 2-ethoxyphenyl | CH | N | CH | CH | S |
| 22 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-dipropoxyphenyl | CH | N | CH | CH | R |

-14-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|--------|---------------|------------------------|----------------------------|----|----|----|----|-----|
| 23 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-dipropoxyphenyl | CH | N | CH | CH | S |
| 24 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,6-dimethoxyphenyl | CH | N | CH | CH | R |
| 25 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,6-dimethoxyphenyl | CH | N | CH | CH | S |
| 26 | ethyl | N-morpholinyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-pentenyl | CH | CH | CH | CH | R |
| 5 27 | ethyl | N-morpholinyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-pentenyl | CH | CH | CH | CH | S |
| 28 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-butenyl | CH | CH | CH | CH | S |
| 29 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-butenyl | CH | CH | CH | CH | R |
| 30 | ethyl | N-morpholinyl | 3,4,5-trimethoxyphenyl | Z-1-(3-pyridyl)-1-pentenyl | CH | CH | CH | CH | R |

-15-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|-----------------------------|--|--------------------------------|------------------------------------|----|----|----|----|-----|
| 31 | ethyl | N-morpho linyl | 3,4,5- trimethoxyp henyl | Z-1-(3- pyridyl)-1- pentenyl | CH | CH | CH | CH | S |
| 32 | E-(1-methyl) ethenyl | trans-4- hydrox y- cycloh exyl | 3,4,5- trimethoxyp henyl | 2,6- dimethoxyph enyl | CH | N | CH | CH | R |
| 33 | E-(1-methyl) ethenyl | trans-4- hydrox y- cycloh exyl | 3,4,5- trimethoxyp henyl | 2,6- dimethoxyph enyl | CH | N | CH | CH | S |
| 34 | ethyl | N-morpho linyl | 3,4,5- trimethoxyp henyl | E-1-(3- pyridyl)-1- hexenyl | CH | CH | CH | CH | R |
| 5 35 | ethyl | N-morpho linyl | 3,4,5- trimethoxyp henyl | E-1-(3- pyridyl)-1- hexenyl | CH | CH | CH | CH | S |
| 36 | propyl | phenyl | 3,4,5- trimethoxyp henyl | E-1-(3- pyridyl)-1- hexenyl | CH | CH | CH | CH | S |

-16-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|---------------------|----------------------------|------------------------|---------------------------------|----|----|----|----|-----|
| 37 | propyl | phenyl | 3,4,5-trimethoxyphenyl | E-1-(3-pyridyl)-1-hexenyl | CH | CH | CH | CH | R |
| 38 | E-(1-methyl)ethenyl | trans-4-hydroxy-cyclohexyl | 4-fluorophenyl | Z-1-(4-methylphenyl)-1-pentenyl | CH | N | CH | CH | S |
| 39 | E-(1-methyl)ethenyl | trans-4-hydroxy-cyclohexyl | 4-fluorophenyl | Z-1-(4-methylphenyl)-1-pentenyl | CH | N | CH | CH | R |
| 40 | E-(1-methyl)ethenyl | trans-4-hydroxy-cyclohexyl | 2-furanyl | 2,6-dimethoxyethyl phenyl | CH | N | CH | CH | S |
| 5 41 | E-(1-methyl)ethenyl | trans-4-hydroxy-cyclohexyl | 2-furanyl | 2,6-dimethoxyethyl phenyl | CH | N | CH | CH | R |

-17-

| # | B | D | G | J | W | X | Y | Z | R/S |
|----|-------------------------|----------------------------|----------------|---------------------------|----|----|----|----|-----|
| 42 | E-(1-methyl) ethenyl | trans-4-hydroxy-cyclohexyl | 4-fluorophenyl | Z-1-cyclohexyl-1-pentenyl | CH | N | CH | CH | S |
| 43 | E-(1-methyl) ethenyl | trans-4-hydroxy-cyclohexyl | 2-furanyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | S |
| 44 | E-(1-methyl) ethenyl | trans-4-hydroxy-cyclohexyl | 2-furanyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |
| 45 | E-(1-methyl) ethenyl | trans-4-hydroxy-cyclohexyl | 2-furanyl | Z-1-cyclohexyl-1-pentenyl | CH | N | CH | CH | S |

-18-

| # | B | D | G | J | W | X | Y | Z | R/S |
|----|-------------------------|---------------------------|-----------------------------|-------------------------------------|----|---|----|----|-----|
| 46 | E-(1-methyl) ethenyl | trans-4-hydroxycyclohexyl | 2-furanyl | Z-1-cyclohexyl-1-pentenyl | CH | N | CH | CH | R |
| 47 | E-(1-methyl) ethenyl | trans-4-hydroxycyclohexyl | 2-furanyl | Z-1-cyclohexyl-3-methoxy-1-propenyl | CH | N | CH | CH | R |
| 48 | E-(1-methyl) ethenyl | trans-4-hydroxycyclohexyl | 2-furanyl | Z-1-cyclohexyl-3-methoxy-1-propenyl | CH | N | CH | CH | S |
| 49 | ethyl | N-morpholinyl | 1,1-dimethyl-2-methoxyethyl | 2,6-diethoxymethyl phenyl | - | S | CH | CH | ND |
| 50 | ethyl | N-morpholinyl | 1,1-dimethyl-2-methoxyethyl | 2,6-diethoxymethyl phenyl | - | S | CH | CH | ND |

5

| # | B | D | G | J | W | X | Y | Z | R/S |
|----|-------------------------|----------------------------|-----------------------------|-----------------------------------|----|----|----|----|-----|
| 51 | E-(1-methyl) ethenyl | trans-4-hydroxy-cyclohexyl | 1,1-dimethyl-2-methoxyethyl | 2,6-dimethoxyethyl phenyl | CH | N | CH | CH | R |
| 52 | E-(1-methyl) ethenyl | 4-(N-methyl)-piperidinyl | t-butyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | S |
| 53 | E-(1-methyl) ethenyl | 4-(N-methyl)-piperidinyl | t-butyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 54 | E-(1-methyl) ethenyl | trans-4-hydroxy-cyclohexyl | 1,1-dimethyl-2-methoxyethyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |
| 55 | E-(1-methyl) ethenyl | 4-(N-methyl)-piperidinyl | 1,1-dimethyl-2-methoxyethyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | S |

-20-

| # | B | D | G | J | W | X | Y | Z | R/S |
|----|-------------------------|--------------------------------|-----------------------------|-------------------------------------|----|----|----|----|-----|
| 56 | E-(1-methyl) ethenyl | 4-(N-methyl) piperidinyl | 1,1-dimethyl-2-methoxyethyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 57 | E-(1-methyl) ethenyl | trans-4-hydroxy- cyclohexyl | 4-(4-hydroxy)pyr anyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |
| 58 | E-(1-methyl) ethenyl | trans-4-hydroxy- cyclohexyl | 1,1-dimethyl-2-methoxyethyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |
| 59 | E-(1-methyl) ethenyl | trans-4-hydroxy- cyclohexyl | isobutyl | Z-1-cyclohexyl-3-methoxy-1-propenyl | CH | N | CH | CH | S |

-21-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|-------------------------|---------------------------|----------------------|-------------------------------------|----|----|----|----|-----|
| 60 | E-(1-methyl) ethenyl | trans-4-hydroxycyclohexyl | isobutyl | 2-1-cyclohexyl-3-methoxy-1-propenyl | CH | N | CH | CH | R |
| 61 | ethyl | N-morpholinyl | 4-pyranyl | 2,6-diethoxymethyl phenyl | - | S | CH | CH | S |
| 62 | ethyl | N-morpholinyl | t-butyl | 2,6-diethoxymethyl phenyl | - | S | CH | CH | R |
| 63 | ethyl | N-morpholinyl | 4-pyranyl | 2,6-diethoxymethyl phenyl | - | S | CH | CH | R |
| 5 64 | ethyl | N-morpholinyl | t-butyl | 2,6-diethoxymethyl phenyl | - | S | CH | CH | S |
| 65 | E-(1-methyl) ethenyl | trans-4-hydroxycyclohexyl | 4-(4-hydroxy)pyranyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |

-22-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|-------------------------|------------------------------|-----------|-----------------------------------|----|----|----|----|-----|
| 66 | ethyl | N-morpholinyl | isobutyl | Z-1-(4-pyranyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 67 | E-(1-methyl) ethenyl | 4-pyridyl | t-butyl | 2,6-diethoxymethyl phenyl | CH | N | CH | CH | R/S |
| 68 | E-(1-methyl) ethenyl | 4-pyridyl | 4-pyranyl | 2,6-diethoxymethyl phenyl | CH | N | CH | CH | R/S |
| 69 | ethyl | N-morpholinyl | isobutyl | Z-1-(4-pyranyl)-1-hexa-1,5-dienyl | - | CH | CH | S | S |
| 5 70 | ethyl | N-morpholinyl | t-butyl | Z-1-(4-pyranyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 71 | E-(1-methyl) ethenyl | 4-(N-methyl)- piperidinyl | isobutyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | S |

-23-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|-------------------------|-----------------------------|--------------------|-----------------------------------|----|----|----|----|-----|
| 72 | E-(1-methyl) ethenyl | 4-(N-methyl) piperidinyl | isobutyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 73 | ethyl | N-morpholinyl | isobutyl | 2,6-diethoxymethyl phenyl | CH | N | CH | CH | R |
| 74 | E-(1-methyl) ethenyl | 4-(N-methyl) piperidinyl | isopropyl | Z-1-cyclohexyl-1-hexenyl | CH | N | CH | CH | S |
| 75 | E-(1-methyl) ethenyl | 4-(N-methyl) piperidinyl | isopropyl | Z-1-cyclohexyl-1-hexenyl | CH | N | CH | CH | R |
| 5 76 | ethyl | N-morpholinyl | 1-methylcyclohexyl | 2,6-diethoxymethyl phenyl | CH | N | CH | CH | S |
| 77 | ethyl | N-morpholinyl | isobutyl | 2,6-diethoxymethyl phenyl | CH | N | CH | CH | S |

-24-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|-------|-------------------|------------------------|---|----|----|----|----|-----|
| 78 | ethyl | N-morpho linyl | 1-methylcyclo hexyl | 2,6-diethoxymet hyl phenyl | CH | N | CH | CH | R |
| 79 | ethyl | N-morpho linyl | 1-methylcyclo hexyl | 2,6-diethoxymet hyl phenyl | - | CH | CH | S | R |
| 80 | ethyl | N-morpho linyl | 1-methylcyclo hexyl | 2,6-diethoxymet hyl phenyl | - | CH | CH | S | S |
| 81 | ethyl | N-morpho linyl | t-butyl | 2,6-diethoxymet hyl phenyl | CH | N | CH | CH | R |
| 5 82 | ethyl | N-morpho linyl | t-butyl | 2-ethoxymethy l-N-indolyl | CH | CH | CH | CH | R |
| 83 | ethyl | N-morpho linyl | isobutyl | 2,6-diethoxymet hyl phenyl | - | CH | CH | S | R |
| 84 | ethyl | N-morpho linyl | isobutyl | 2,6-diethoxymet hyl phenyl | - | CH | CH | S | S |
| 85 | ethyl | N-morpho linyl | 1-methylcyclo hexyl | Z-1-(3-pyridyl)-1- hexa-1,5- dienyl | - | CH | CH | S | R |

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|--------|---------------|------------------------|-----------------------------------|----|----|----|----|-----|
| 86 | ethyl | N-morpholinyl | 1-methylcyclohexyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |
| 87 | ethyl | N-morpholinyl | 1-methylcyclohexyl | 2-1-(4-pyranyl)-1-hexa-1,5-dienyl | - | CH | CH | S | S |
| 88 | propyl | phenyl | 3,4,5-trimethoxyphenyl | 2,5-diethoxyphenyl | CH | N | CH | CH | S |
| 89 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2,6-diethoxymethylphenyl | CH | N | CH | CH | ND |
| 5 90 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | ND |
| 91 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-1-(4-pyranyl)-1-hexa-1,5-dienyl | - | CH | CH | S | ND |
| 92 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | E-1-(3-pyridyl)-1-pentenyl | CH | CH | CH | CH | ND |
| 93 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2,6-diethoxymethylphenyl | - | CH | CH | S | R |
| 94 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |

-26-

| # | B | D | G | J | W | X | Y | Z | R/S |
|------|---------------------|---------------|-------------------------------------|-----------------------------------|----|----|----|----|-----|
| 95 | ethyl | N-morpholinyl | 1-hydroxycyclohexyl | Z-1-(4-pyranyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 96 | ethyl | N-morpholinyl | 1-methylcyclohexyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | R |
| 97 | ethyl | N-morpholinyl | t-butyl | 2-ethoxymethyl-N-indolyl | CH | CH | CH | CH | R |
| 98 | E-(1-methyl)ethenyl | 4-pyridyl | 1,1,2-trimethylpropyl | Z-1-cyclohexyl-1-pentenyl | CH | N | CH | CH | R |
| 5 99 | E-(1-methyl)ethenyl | 4-pyridyl | cis-(4-methoxy-1-hydroxy)cyclohexyl | Z-1-cyclohexyl-1-pentenyl | CH | N | CH | CH | ND |
| 100 | E-(1-methyl)ethenyl | 4-pyridyl | cis-(4-methoxy-1-hydroxy)cyclohexyl | Z-1-cyclohexyl-1-pentenyl | CH | N | CH | CH | ND |
| 101 | ethyl | N-morpholinyl | 5-methoxymethyl-2-methylphenyl | Z-1-(3-pyridyl)-1-hexa-1,5-dienyl | - | CH | CH | S | S |

-27-

| # | B | D | G | J | W | X | Y | Z | R/S |
|-------|-------------------------|---------------|---|---------------------------|----|----|----|----|-----|
| 102 | ethyl | N-morpholinyl | 5-methoxymethyl-2-methylphenyl | 2,6-diethoxymethyl phenyl | - | CH | CH | S | R |
| 103 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 104 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | S |
| 105 | ethyl | N-morpholinyl | (1R, 2R)-2-methylcyclohexyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 5 106 | E-(1-methyl) ethenyl | 4-pyridyl | 1,1,2-trimethylpropyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 107 | ethyl | N-morpholinyl | (R,R)-5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl-2-enyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 108 | E-(1-methyl) ethenyl | 4-pyridyl | (1R, 2R)-2-methylcyclohexyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | ND |

-28-

| # | B | D | G | J | W | X | Y | Z | R/S |
|-------|-------------------------|---------------|--|-------------------------------------|----|----|----|----|-----|
| 109 | E-(1-methyl) ethenyl | 4-pyridyl | 1,1,2-trimethylpropyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | ND |
| 110 | ethyl | N-morpholinyl | (R,R,R)-5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 111 | ethyl | N-morpholinyl | (R,R,R)-5-ethoxy-2-methylcyclohexyl | 2,5-dipropyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 112 | ethyl | N-morpholinyl | (R,R,R)-5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl | 2-methyl-5-butyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 5 113 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-3-(1-methoxy)-2-hexenyl | CH | N | CH | CH | R |
| 114 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-3-(1-methoxy)-2-hexenyl | CH | N | CH | CH | S |
| 115 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-3-(1-methoxy)-4-methyl-2-pentenyl | CH | N | CH | CH | R |

| # | B | D | G | J | W | X | Y | Z | R/S |
|-------|-------|---------------|--|-------------------------------------|----|----|----|----|-----|
| 116 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | Z-3-(1-methoxy)-4-methyl-2-pentenyl | CH | N | CH | CH | S |
| 117 | ethyl | N-morpholinyl | (1R, 2R)-2-methylcyclohexyl | Z-3-(1-methoxy)-2-hexenyl | CH | N | CH | CH | R |
| 118 | ethyl | N-morpholinyl | (1R, 2R)-2-methylcyclohexyl | Z-3-(1-methoxy)-4-methyl-2-pentenyl | CH | N | CH | CH | R |
| 119 | ethyl | N-morpholinyl | (2S, 4R)-4-ethoxy-N-aceto-2-pyrrolidiny 1 | Z-3-(1-methoxy)-2-hexenyl | CH | N | CH | CH | R |
| 5 120 | ethyl | N-morpholinyl | (1R, 2R)-2-methylcyclohexyl | 2-methyl-5-butyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 121 | ethyl | N-morpholinyl | (R,R,R)-5-ethoxy-2-methylcyclohexyl | 2-methyl-5-butyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 122 | ethyl | N-morpholinyl | 1,1,2-trimethylpropyl | 2-methyl-5-butyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 123 | ethyl | N-morpholinyl | (R,R,R)-5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl | Z-3-(1-methoxy)-2-hexenyl | CH | N | CH | CH | R |

-30-

| # | B | D | G | J | W | X | Y | Z | R/S |
|-----|-------|---------------|--|-------------------------------------|----|----|----|----|-----|
| 124 | ethyl | N-morpholinyl | (R,R,R)-5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl | Z-3-(1-methoxy)-4-methyl-2-pentenyl | CH | N | CH | CH | R |
| 125 | ethyl | N-morpholinyl | (R,R,R)-5-ethoxy-2-methylcyclohexyl | Z-3-(1-methoxy)-2-hexenyl | CH | N | CH | CH | ND |
| 126 | ethyl | N-morpholinyl | (R,R,R)-5-isopropyl-2-methylcyclohexyl | 2-methyl-5-butyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 127 | ethyl | N-morpholinyl | (R,R,R)-5-ethoxy-2-methylcyclohexyl | 2,5-dimethyl-N-pyrrolyl | CH | CH | CH | CH | R |
| 128 | ethyl | N-morpholinyl | (1R, 2R)-2-methylcyclohexyl | Z-3-(2-methyl)-3-heptenyl | CH | N | CH | CH | R |
| 129 | ethyl | N-morpholinyl | (R,R,R)-5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl | Z-3-(2-methyl)-3-heptenyl | CH | N | CH | CH | R |

5

-31-

| # | B | D | G | J | W | X | Y | Z | R/S |
|-----|-------------------------------------|-------------------|--|------------------------------------|----|---|----|----|-----|
| 130 | ethyl | N-morpho linyl | (R,R,R)-5- ethoxy-2- methylcyclo hexyl | Z-3- (2- methyl)-3- heptenyl | CH | N | CH | CH | R |
| 131 | E-(1- methyl) etheny l | 4- pyrany l | (1R, 2R)-2- methylcyclo hexyl | Z-3-(1- methoxy)-2- hexenyl | CH | N | CH | CH | R/S |
| 132 | ethyl | N-morpho linyl | (2S, 4R)-4- ethoxy-N- aceto-2- pyrrolidiny l | Z-3-(2- methyl)-3- heptenyl | CH | N | CH | CH | R |
| 133 | ethyl | N-morpho linyl | (R,R,R)-5- (1-methyl- 1- methoxyethy l)-2- methylcyclo hexyl | Z-2-(2- hexenyl) | CH | N | CH | CH | R |

5 The compounds of this invention may be obtained
 using any conventional technique. Preferably, these
 compounds are chemically synthesized from readily available
 starting materials, such as alpha-amino acids. Modular and
 convergent methods for the synthesis of these compounds are
 10 also preferred. In a convergent approach, for example, large
 sections of the final product are brought together in the
 last stages of the synthesis, rather than by incremental
 addition of small pieces to a growing molecular chain.

-32-

Various synthesis schemes for these compounds are presented in the examples of this application.

The compounds utilized in the compositions and methods of this invention may be modified by appending
5 appropriate functionalities to enhance selective biological properties. Such modifications are known in the art and include those which increase biological penetration into a given biological system (e.g., blood, lymphatic system, central nervous system), increase oral availability, increase
10 solubility to allow administration by injection, alter metabolism and alter rate of excretion.

The compounds of this invention are characterized by the ability to increase, restore or maintain the sensitivity of MDR cells to cytotoxic compounds, such as, for
15 example, those typically used in chemotherapy. Based on that ability, the compounds of this invention are advantageously used to increase the effectiveness of chemotherapy in individuals who are afflicted with drug-resistant cancers, tumors, metastases or disease. In addition, the compounds of
20 this invention are capable of maintaining sensitivity to therapeutic or prophylactic agents in non-resistant cells. Therefore, the compounds of this invention are useful in treating or preventing multi-drug resistance ("MDR") in a patient. More specifically, these compounds are useful in
25 treating or preventing P-glycoprotein-mediated MDR and MRP-mediated MDR.

The compounds of this invention are also characterized by their ability to stimulate neurite growth through their binding affinity for FKBP-12. Based on that
30 ability, the compounds of this invention may be used to stimulate nerve growth in a patient who has suffered nerve damage as a result of trauma or disease.

As used throughout this application, the term "patient" refers to mammals, including humans.

-33-

Multi-drug resistance has been explained in the art thus far by the presence of two independent proteins within the cell - the MDR1 P-glycoprotein or the MRP protein. Methods for quantitating the effectiveness of the compounds of this invention towards restoring drug sensitivity caused by either protein are known. For example, any assay known to measure the restoration of the anti-proliferative activity of a drug may be employed to test the compounds of this invention. These assays utilize cell lines resistant to particular drugs, and characterized by the presence of one or both of MDR1 and MRP. These cell lines include HL60/ADR, L1210, P338D, CHO and MCF7. The cell line is then exposed to compounds of this invention in the presence or absence of the drug to which it is resistant, such as doxorubicin.

The neurotrophic activity of the compounds of this invention is directly related to their affinity for FKBP12 and their ability to inhibit FKBP12 rotomase activity. In order to candidate these properties, several assays known in the art may be employed. For example, competitive LH20 binding assays using labelled FK-506 as a reporting ligand have been described by M. W. Harding et al., Nature, 341, pp. 758-60 (1989) and by J. J. Siekierka et al., Nature, 341, pp. 755-57 (1989).

Preferably, the assay measures inhibition of FKBP12 rotomase activity. Such an assay has also been described by M. W. Harding et al., supra and by J. J. Siekierka et al., supra. In this assay the isomerization of an artificial substrate -- N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide -- is followed spectrophotometrically. The assay includes the cis form of the substrate, FKBP12, the inhibitor and chymotrypsin. Chymotrypsin is able to cleave p-nitroanilide from the trans form of the substrate, but not the cis form. Release of p-nitroanilide is measured.

-34-

According to another embodiment, the invention provides pharmaceutical compositions comprising the compounds of this invention or pharmaceutically acceptable derivatives thereof in an amount effective for the treatment or
5 prevention of multi-drug resistance. According to another embodiment, the invention provides pharmaceutical compositions comprising the compounds of this invention or pharmaceutically acceptable derivatives thereof in an amount effective for stimulating neurite growth. A
10 "pharmaceutically acceptable derivative" denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of a compound of this invention or any other compound which, upon administration to a patient, is capable of providing (directly or indirectly) a compound of this
15 invention.

Pharmaceutically acceptable salts derived from inorganic or organic acids and bases. Included among such acid salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate,
20 citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate,
25 maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenyl-propionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate. Base salts include ammonium salts, alkali metal salts, such
30 as sodium and potassium salts, alkaline earth metal salts, such as calcium and magnesium salts, salts with organic bases, such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be

-35-

quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates, such as dimethyl, diethyl, dibutyl and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides, such as benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The pharmaceutical compositions of this invention further comprise a pharmaceutically acceptable carrier, adjuvant or vehicle. Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethyl cellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

According to this invention, the pharmaceutical compositions may be in the form of a sterile parenteral preparation, for example a sterile injectable aqueous or oleaginous suspension. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques.

-36-

This parenteral preparation may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as do natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as Ph. Hely or similar alcohol.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These can be

-37-

prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

For ophthalmic use, the pharmaceutical compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline, or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without

-38-

a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the pharmaceutical compositions may be formulated in an ointment such as petrolatum.

5 The pharmaceutical compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or
10 other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

 The amount of active ingredient that may be combined with the carrier materials to produce a single
15 dosage form will vary depending upon the host treated, and the particular mode of administration. It should be understood, however, that a specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the activity of the specific compound
20 employed, the age, body weight, general health, sex and diet of the patient, the time of administration and rate of excretion of the compound, the particular drug combination, and the judgment of the treating physician and the severity of the particular disease being treated. The amount of
25 active ingredient may also depend upon the therapeutic or prophylactic agent, if any, with which the ingredient is co-administered.

 According to a preferred embodiment, the compounds of this invention are administered in combination with a
30 therapeutic agent or a neurotrophic agent, depending upon the desired use.

 For example, in order to increase the susceptibility of the MDR cells within the patient, the compounds of this invention may be administered with one or

-39-

more chemotherapeutic agents, such as actinomycin D, doxorubicin, vincristine, vinblastine, etoposide, amsacrine, mitoxantrone, tenipaside, taxol or colchicine. For the same purpose, the compounds of this invention may also be used in
5 combination with a chemosensitizing agent, such as cyclosporin A and its analogs, phenothiazines or thioxantheres. As used in this application, the term "chemosensitizing agent, excludes the compounds of this invention.

10 For neurotrophic use, the compounds of this invention may be combined with other neurotrophic factors, such as nerve growth factor (NGF), insulin growth factor (IGF-1) and its active truncated derivatives such as gIGF-1, acidic and basic fibroblast growth factor (aFGF and bFGF,
15 respectively), platelet-derived growth factors (PDGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factors (CNTF), glial cell line-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4/5). As used in this application, the term "neurotrophic factor"
20 excludes the FKBP12 binding compounds described herein, as well as FK-506 and rapamycin.

The additional agent may be part of a single pharmaceutical composition or may be separately administered sequentially or concurrently to the patient. Thus, according
25 to one preferred embodiment, the pharmaceutical compositions of this invention comprise a compound of this invention and a chemosensitizing agent. According to another preferred embodiment, the pharmaceutical compositions of this invention comprise a compound of this invention and a chemotherapeutic
30 agent. According to yet another preferred embodiment, the pharmaceutical compositions of this invention comprise a compound of this invention and a neurotrophic agent.

According to another embodiment, the invention provides methods for treating or preventing multi-drug

-40-

resistance in a patient by administering a composition of this invention. Effective dosage levels for treating or preventing MDR range from between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 50 mg/kg body weight per day of a compound of this invention. A typical composition for use in treating MDR will contain between about 5% and about 95% of active compound(s) (w/w), whether it be solely one of the compounds of this invention or a combination of a compound of this invention and another chemotherapeutic or chemosensitizing agent. Preferably, such preparations contain between about 20% and about 80% active compound(s).

The amount of chemosensitizing or chemotherapeutic agent used in combination with the compounds of this invention, whether part of the same composition or separately administered, will be less than that used in a monotherapy. Preferably, the amount of chemosensitizing or chemotherapeutic agent is less than 80% of the dosage used in a monotherapy. Monotherapeutic dosages of such agents are known in the art.

In another embodiment, the invention provides methods for stimulating neurite outgrowth by administering a composition of this invention. The amount of the compound and, optionally, a neurotrophic factor that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated, the particular mode of administration. The two active ingredients of the pharmaceutical compositions of this invention act synergistically to stimulate neurite outgrowth.

In the treatment of multi-drug resistance or stimulation of neurite outgrowth, dosage levels of between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 50 mg/kg body weight per day of the active ingredient compound are useful. A

-41-

typical preparation will contain between about 5% and about 95% active compound (w/w). Preferably, such preparations contain between about 20% and about 80% active compound.

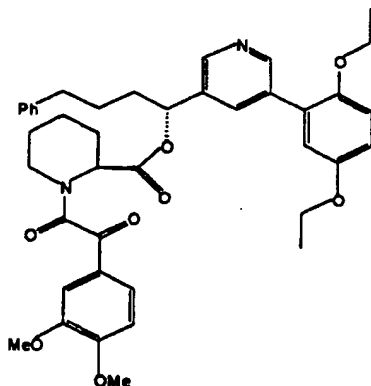
The amount of the neurotrophic factor in these
5 compositions and combination therapies will be less than that required in a monotherapy utilizing only that neurotrophic factor. Preferably, the compositions should be formulated so that a dosage of between 0.01 - 100 mg/kg body weight/day of the compounds of this invention can be administered. The
10 neurotrophic factor, whether part of the same composition or separately administered, should be given at a dosage of between 0.01 - 100 µg/kg body weight/day.

The neurotrophic methods and compositions of this invention may be used to treat nerve damage caused by a wide
15 variety of diseases or physical traumas. These include, but are not limited to, Alzheimer's disease, Parkinson's disease, ALS, stroke and ischemia associated with stroke, neural paropathy, other neural degenerative diseases, motor neuron diseases, sciatic crush, spinal cord injuries and facial
20 nerve crush.

In order that this invention may be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any
25 way.

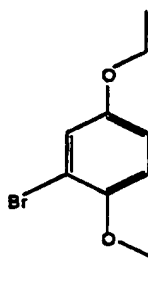
-42-

Example 1 -- Preparation of Compound 11



11

To a solution of bromohydroquinone (10.0g, 0.053mol) in DMF (70mL) was added 1-bromopropane (39.0g, 0.318mol) and cesium carbonate (50g, 0.153mol) and heated at 90°C for 2 days. The reaction mixture was filtered through Celite and washed with ethyl acetate (500mL). The solution was concentrated and chromatographed on silica gel eluting with 0.25 % ethyl acetate-hexane to yield 9.0g(62%) of the bromobenzene (136) as a colorless oil; ¹H NMR (500MHz, CDCl₃) (136) δ 7.08(d,1H), 6.80 (dd,1H), 6.77(dd,1H), 4.00(q,2H), 3.93(q,2H), 1.40(t,3H), 1.38(t,3H).

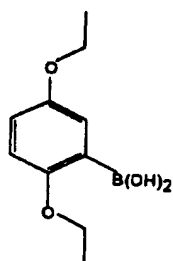


136

To a solution of the bromobenzene (136) (6.6g, 24.2mmole) in THF (50mL) was added dropwise n-BuLi in hexane

-43-

- (1.6M, 33ml, 52.9mmole) at -78°C under a N_2 atmosphere, and stirred at the same temperature for 1hr. The resulting mixture was treated with trimethylborate (9.0ml, 79.5mmole) at -78°C and allowed to warm to room temperature over 3 hrs.
- 5 Aqueous HCl (100mL, 7%) was added to the reaction mixture and stirred overnight at room temperature. The aqueous layer was washed with CH_2Cl_2 . All the organics were combined and dried over MgSO_4 . The solution was concentrated and chromatographed on silica gel eluting with 0.5% methanol- CH_2Cl_2 to 2%
- 10 methanol- CH_2Cl_2 to yield the boronic acid (137) (4.3 g, 75%) as a colorless solid; ^1H NMR (500 MHz, CDCl_3) (137) δ 7.38(d,1H), 6.94(dd,1H), 6.81(d,1H), 6.46(brs,2H), 4.08(q,2H), 4.02(q,2H).

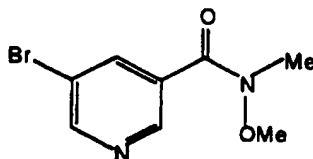


137

- 15 To a solution of 5-bromonicotinic acid (5.0g, 24.8mmole) in a mixture of CH_2Cl_2 (50 mL) and DMF (5mL) was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (5.21g, 27.2mmole), N,O-dimethyl-hydroxylamine hydrochloride (2.66g, 27.2mmole) and diisopropylethyl-amine
- 20 (4.74ml, 27.2mmole), and the mixture stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (200mL) and washed with water (100mlx3). The organic phase was dried over MgSO_4 and concentrated. The crude oil was chromatographed on silica gel eluting with 33% ethyl
- 25 acetate-hexanes to yield the bromopyridine (138) (4.5g, 74%) as a colorless oil; ^1H NMR (500MHz, CDCl_3) (138) δ

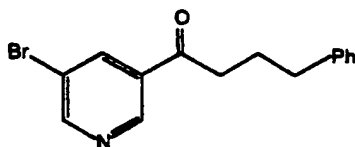
-44-

8.73 (dd, 1H), 8.62 (dd, 1H), 8.08 (dd, 1H), 3.47 (s, 3H),
3.27 (s, 3H).



138

To a solution of the amide (138) (4.2 g, 17.1mmole)
5 in THF (40 mL) was added dropwise phenylpropylmagnesium
bromide (0.5 M, 70 ml, 35.0mmole) prepared from 1-bromo-3-
phenylpropane and magnesium at 0°C for 2hrs. The reaction
mixture was quenched with aqueous NH₄Cl (5mL), and extracted
with ethyl acetate (300mL). The organics were dried over
10 MgSO₄, concentrated and chromatographed on silica gel eluting
with 20% ethyl acetate-hexanes to yield the ketone (139) (2.34
g, 45%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) (139) δ
8.98 (dd, 1H), 8.80 (dd, 1H), 8.30 (dd, 1H), 7.40-7.12 (m, 5H),
2.92 (t, 2H), 2.70 (t, 2H), 2.10 (tt, 2H).

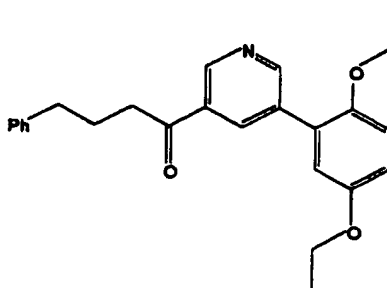


139

15 To a stirred mixture of the bromopyridine (139)
(304mg, 1.0mmole) and tetrakis(triphenylphosphine)-
palladium(0) (30mg, 0.024mmole) in toluene (30mL) was
successively added the boronic acid (137) (420mg, 2mmole)
20 dissolved in 2ml of ethanol and sodium carbonate (420 mg,
4mmole) dissolved in 2ml of H₂O. The resulting solution was
heated at reflux for 2 hrs, and allowed to cool to room
temperature. The solution was diluted with ethyl acetate
(100mL), and the separated organic phase was dried over

-45-

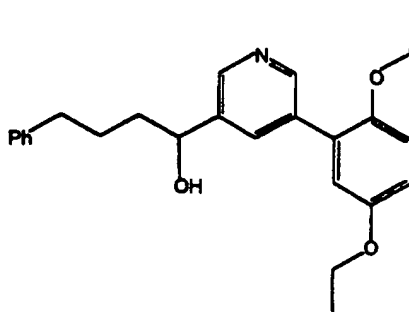
MgSO₄. The solution was concentrated and chromatographed on silica gel eluting with 20% ethyl acetate-hexanes to yield the biaryl (140) (215 mg, 55%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) (140) δ 9.04 (d, 1H), 8.93 (d, 1H), 8.39 (dd, 1H), 7.30-7.22 (m, 2H), 7.21-7.14 (m, 3H), 6.97-6.85 (m, 3H), 4.02 (q, 2H), 3.96 (q, 2H), 2.99 (t, 2H), 2.74 (t, 2H), 2.12 (tt, 2H), 1.40 (t, 3H), 1.28 (t, 3H).



140

To a solution of the ketone (140) (210 mg, 0.54mmole) in methanol (10mL) at 0°C was added sodium borohydride (30mg, 0.79mmole), and the mixture stirred at room temperature for 20 min. The reaction mixture was quenched with water (1mL) and concentrated. The residual oil was extracted with CH₂Cl₂ and the organic phase was dried over MgSO₄. The solution was concentrated and chromatographed on silica gel eluting with 50% ethyl acetate-hexanes to yield the alcohol (141) (157mg, 74%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) (141) δ 8.60 (d, 1H), 8.39 (d, 1H), 7.90 (dd, 1H), 7.27-7.19 (m, 2H), 6.90-6.80 (m, 5H), 4.75-4.68 (m, 1H), 3.98 (q, 2H), 3.90 (q, 2H), 3.20 (brs, 1H), 2.67-2.57 (m, 2H), 1.91-1.58 (m, 4H), 1.39 (t, 3H), 1.24 (t, 3H).

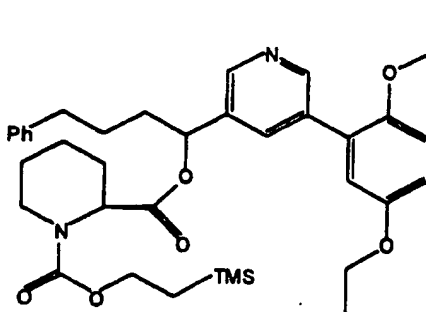
-46-



141

To a solution of the alcohol (141) in CH_2Cl_2 (10mL) was added (S)-N-2-trimethylsilylethoxycarbonyl-pipecolinic acid (210mg, 0.77mmole), N-ethyl-N'-1-(3-dimethylaminopropyl)-carbodiimide hydrochloride (150mg, 0.77mmole), and dimethylaminopyridine (3mg, 0.025mmole). The resulting mixture was stirred at room temperature for 14 hrs, and quenched by adding H_2O (5mL). The mixture was diluted with CH_2Cl_2 (20 mL). The organic phase was dried over MgSO_4 . The solution was concentrated and chromatographed on silica gel eluting with 20% ethyl acetate-hexane to yield the ester (142) (230mg, 93%) as a colorless oil; ^1H NMR (500 MHz, CDCl_3) (142) δ 8.70(d, 1H), 8.48(d, 1H), 7.80(dd, 1H), 7.28-7.18(m, 2H), 7.17-7.07(m, 3H), 6.93-6.82(m, 5H), 5.90-5.80(m, 1H), 4.98-4.92 and 4.82-4.72(m, 1H), 4.22-3.84(m, 6H), 3.02-2.83(m, 1H), 2.60(t, 2H), 2.28-2.12(m, 1H), 2.07-1.91(m, 1H), 1.90-1.80(m, 1H), 1.78-0.78(m, 16H), 0.05-0.15(m, 9H).

-47-



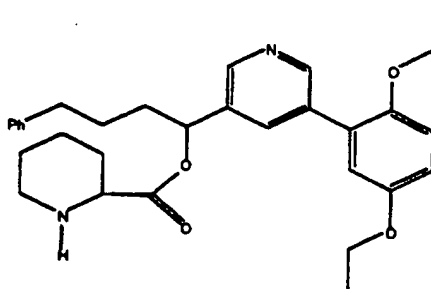
142

To a solution of the ester (142) (220mg, 0.34mmole) in acetonitrile (15mL) was added cesium fluoride (800mg, 5.26mmole) and the suspension heated at reflux for 14 hrs.

5 The reaction mixture was allowed to cool to room temperature and diluted with CH_2Cl_2 (100mL). The mixture was vacuum filtered through Celite and washed with CH_2Cl_2 . The solution was concentrated and chromatographed on silica gel eluting with 2% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ to 5% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ to yield the amine (143)

10 (85mg, 50%) as a colorless oil; ^1H NMR (500 MHz, CDCl_3) (143) δ 8.70(d, 1H), 8.48(d, 1H), 7.82(dd, 1H), 7.28-7.20(m, 2H), 7.19-7.08(m, 3H), 6.92-6.81(m, 3H), 5.88-5.82(m, 1H), 4.06-3.98(m, 2H), 3.97-3.89(m, 2H), 3.41-3.31(m, 1H), 3.08-3.01(m, 1H), 2.69-2.55(m, 2H), 2.10-1.32(m, 15H), 1.30-

15 1.21(m, 3H).



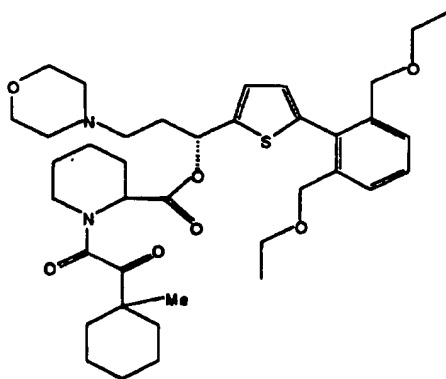
143

To a solution of the amine (143) (40mg, 0.080mmole) in CH_2Cl_2 (5mL) was added 3,4-dimethoxybenzoylformic acid

-48-

(85mg, 0.405 mmole) and N-ethyl-N'-1-(3-dimethylaminopropyl)-carbodiimide hydrochlorid (80 mg, 0.417 mmole). The resulting mixture was stirred at room temperature for 5 days, and quenched by adding H₂O (5mL). The mixture was diluted
5 with CH₂Cl₂ (20 mL), the layers separated and the organic phase was dried over MgSO₄. The solution was concentrated and chromatographed on silica gel eluting with 0.5% MeOH-CH₂Cl₂ to 1.0% MeOH-CH₂Cl₂ to yield 11 (6.8mg, 24%) and the diastereomer of compound 11 (8.9mg, 32%) as a colorless oils;
10 TLC R_f=0.20 (2.5% CH₂Cl₂/MeOH) (Compound 11), R_f=0.23 (2.5% CH₂Cl₂/MeOH) (The diastereomer of compound 11); ¹H NMR (500 MHz, CDCl₃) (11) δ 8.72 and 8.68(d,1H), 8.53 and 8.36(d,1H), 7.87 and 7.74(m,1H), 7.63-7.06(m,8H), 6.87(m,3H), 5.95 and 5.80(dd,1H), 6.40(m,1H), 4.07-3.82(m,10H),
15 3.48(m,1H), 3.18 and 2.96(dt,1H), 2.65 and 2.58(t,2H), 2.40 and 2.20(m,1H), 2.15-1.19(m,15H).

Example 2 -- Preparation of Compound 79

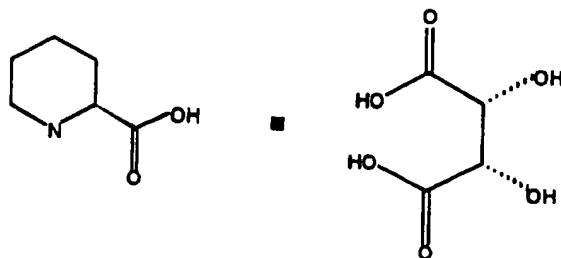


79

To a boiling mixture of 300g (2.32 mole) of D,L-
20 pipecolic acid in methanol (1.23L) was added 348g (2.32 mole) of D-Tartaric acid. The solution was allowed to stir for five minutes and then cooled to room temperature. The salt was filtered and washed with methanol and was purified

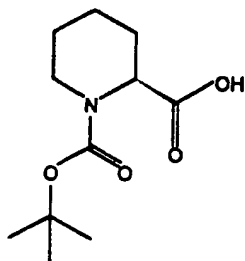
-49-

by recrystallization two times from water/acetone to give 124.2g of 144.



144

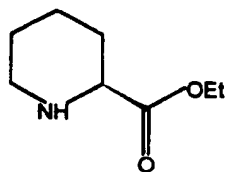
To a stirred mixture of 124.2g (0.445 mole) of 144 and 340ml (1.95 mole) of N,N-diisopropylethylamine at 0°C was added 282ml (2.23 mole) of chlorotrimethyl-silane dropwise. The mixture was allowed to warm to room temperature and stir for one hour. The mixture was re-cooled to 0°C and treated with 85ml (0.49 mole) of N,N-diisopropylethylamine followed by the dropwise addition of 102g (0.467 mole) of di-t-butylidicarbonate dissolved in methylene chloride (270ml). The reaction was allowed to warm to room temperature and stir overnight. The solvent was evaporated under reduced pressure, basified with 1N aqueous NaOH, and washed with ether. The aqueous phase was acidified with aqueous KHSO₄, extracted with ether, and the organic phase dried over MgSO₄. The solvent was evaporated under reduced pressure and purified by recrystallization from ethyl acetate/hexanes to give 76g of optically pure 145.



145

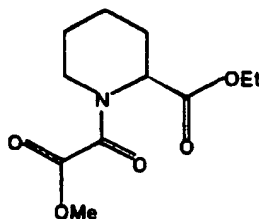
-50-

A solution of 3.6g (15.7 mmole) of 145 in ethanol (30ml) was cooled to 0°C, subjected to a stream of HCl(g) for fifteen minutes, and allowed to stir for 48hr at room temperature. The solvent was evaporated under reduced pressure and the resulting residue dissolved in methylene chloride. This solution was washed two times with NaHCO₃(aq) and once with brine, dried over Na₂SO₄ and the solvent was evaporated under reduced pressure to give 2.5g of 146.



146

To a stirred solution of 2.5g (14.0 mmole) of 146 and 3.4 ml (19.1 mmole) of N,N-diisopropylethylamine at 0°C was added 1.6ml (17.5 mmole) of methyloxalyl chloride. The mixture was allowed to stir for thirty-five minutes, diluted with methylene chloride, washed once with water, once with brine, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure and purified by chromatography on silica gel using 9:1 hexane:ethyl acetate as eluent to give 3.52g of 147.



147

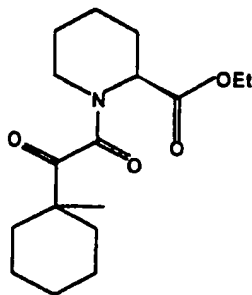
LiBr (12.3g, 142mmole) was dissolved into 1-Methylcyclohexanol (10.8g, 94.6mmole) with gentle heating.

-51-

To this was added 5mL of 48% HBr, the mixture was cooled to 0°C and a further 17mL HBr(aq) added in a dropwise fashion. This was stirred for 3.5h at which point the upper layer was separated, washed with ethylene glycol, diluted with ether and then dried over Na₂SO₄. The filtered solution was
5 carefully concentrated to yield 12.4g (70mmole, 74%) of 1-bromo-1-methylcyclohexane (148) as a light yellow oil, greater than 95% pure by tlc and ¹H NMR.

Bromide 148 (8.0g, 45.2mmole) was dissolved in 20mL
10 of THF and treated with 1.14g (47.4mmole) of Mg° turnings. The reaction was heated to 55°C, following initiation with 2 drops of 1,2-dibromoethane, for a total of 90 mins. Cooled to ambient temperature and used as Methylcyclohexyl Grignard (149).

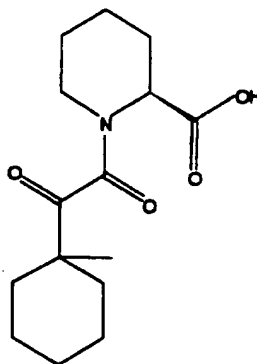
15 The methyl oxamide (147, 1.5g, 6.17mmole), dissolved in 10mL of CH₂Cl₂ at ambient temperature, was treated dropwise with portions of the grignard solution as prepared above. The reaction progress, as noted by conversion of starting material to the desired product, was
20 monitored by tlc. Complete consumption of starting material required 5-6 eq's of the grignard reagent. The reaction was quenched by addition of 10% KHSO₄ and extracted with CH₂Cl₂. The organics were washed with water and brine, dried over MgSO₄ and concentrated. Elution from silica gel with 10%
25 ethyl acetate in hexanes gave product (150) (712mg, 37%) pure by tlc and ¹H NMR.



150

-52-

The ethyl ester 150 (575mg, 1.86mmole) was dissolved in 4mL of THF at 0°C and treated in a slow, dropwise fashion with 2 eq's of 1N LiOH. Brought to ambient temperature after 30mins and allowed to stir overnight. The reaction was acidified with 10% KHSO₄, extracted with CH₂Cl₂, the organics washed with brine, dried over Na₂SO₄ and stripped to yield 510mg (1.81mmole, 98%) of acid 151, pure by ¹HNMR and containing less than 0.5% of the epimer as determined by HPLC analysis.

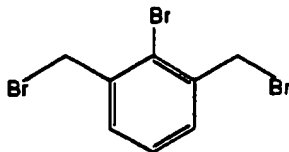


10

151

To a solution of 2-bromo-m-xylene (157g, 0.849mol) in carbon tetrachloride (1.5L) was added N-bromosuccinimide (362g, 2.034mol) and benzoyl peroxide (1.38g, 7.75mmole), and heated at reflux for 3hrs. The reaction mixture was allowed to cool to room temperature and washed with H₂O (500ml X 2), dried over MgSO₄ and concentrated. The obtained solid was washed with n-hexane(1L) and recrystallized from i-PrOH to give the tribromide(152) as a colorless solid (98g, 34%); ¹H NMR (500 MHz, CDCl₃) (152) δ 7.40(d,2H), 7.26(dd,1H), 4.62(s,4H).

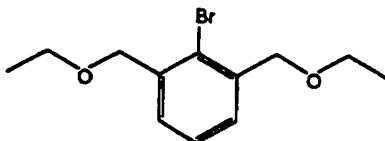
20



152

-53-

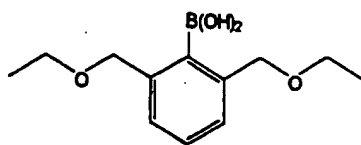
To a suspension of sodium hydride (18.6 g as an 80% dispersion in mineral oil, 0.62 mol) in anhydrous THF (80 mL) was added ethanol (34.1 mL, 0.583 mol) at 0°C and stirred for 30 min. To the mixture was added tribromide (152) (33.0 g, 0.096 mol) and heated at 45°C for 2 hrs. The reaction mixture was allowed to cool to room temperature and diluted with ethyl acetate (200 mL). The solution was washed with H₂O and the organics dried over MgSO₄. The solution was concentrated and chromatographed on silica gel eluting with 30% CH₂Cl₂-hexanes to yield the diethylester (153) (25.0 g, 95%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) (153) δ 7.40 (d, 2H), 7.30 (dd, 1H), 4.58 (s, 4H), 3.60 (q, 4H), 1.28 (t, 6H).



153

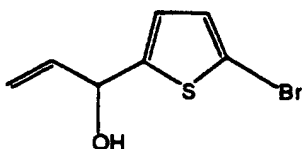
To a solution of the bromobenzene (153) (25.0 g, 91.6 mmole) in THF (250 mL) was added dropwise n-BuLi solution in hexane (1.6 M, 63 mL, 101 mmole) at -78°C under N₂ atmosphere, and stirred at the same temperature for 2 hr. To the resulting mixture was added trimethyl borate (34 mL, 0.3 mol) over 10 min at -78°C, and stirred for 2 hrs. The reaction mixture was allowed to warm to room temperature over 1 hr and stirred overnight. Aqueous 4 N HCl (300 mL) was added to the reaction mixture and stirred for 4 hrs. The mixture was concentrated to ca. 250 mL and extracted with CH₂Cl₂ (300 mL X 3). The organics were extracted with 2N NaOH (200 mL X 2). The combined aqueous layers were washed with CH₂Cl₂ and acidified to pH 2 with 6N HCl. The mixture was extracted with CH₂Cl₂, dried over MgSO₄ and concentrated to yield the boronic acid (154) (10.5 g, 48%) as a colorless solid; ¹H NMR (500 MHz, CDCl₃) (154) δ 7.58 (s, 2H), 7.33 (dd, 1H), 7.27 (d, 2H), 4.50 (s, 4H), 3.58 (q, 4H), 1.22 (t, 6H).

-54-



154

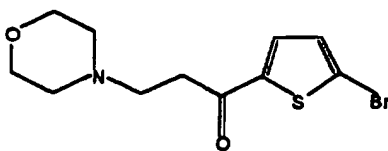
To a solution of 5-bromo-2-thiophenecarboxaldehyde (10.0g, 52.4mmole) in anhydrous THF (100 mL) was slowly added vinylmagnesium bromide (1.0M solution in THF, 60.0ml, 60.0mmole) at -78°C and stirred for 1 hr. The reaction mixture was quenched by addition of sat. aqueous NH₄Cl (10 mL), and allowed to warm to room temperature. The mixture was diluted with ethyl acetate (200mL) and dried over MgSO₄ and concentrated. The obtained oil was chromatographed on silica gel eluting with 10% ethyl acetate-hexane to yield the alcohol (155) (6.0 g, 52%) as a colorless oil.



155

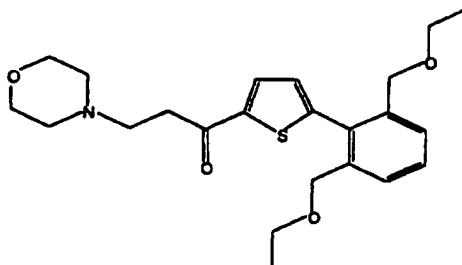
To a solution of the alcohol (155) (2.80g, 12.6mmole) in CH₂Cl₂ (100mL) was added manganese(IV) oxide (5.0g, 57.5mmole) and morpholine (92.3ml, 26.4mmole) and the suspension was stirred at room temperature for 14 hrs. The mixture was vacuum filtered through Celite and washed with CH₂Cl₂. The solution was concentrated and chromatographed on silica gel eluting with 10% ethyl acetate-hexanes to yield the ketone (156) (3.5g, 91%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) (156) δ 7.43(d,1H), 7.09(d,1H), 3.72-3.62(m,4H), 3.00(t,2H), 2.78(t,2H), 2.55-2.40(m,4H).

-55-



156

To a stirred mixture of the bromothiophene (156) (2.0g, 6.6mmole) and tetrakis(triphenylphosphine)-
5 palladium(0) (500mg, 0.402mmole) in toluene (100mL) was successively added the boronic acid (154) (2.57g, 10.8mmole) dissolved in 7mL of ethanol and sodium carbonate monohydrate (2.74 g, 22.1 mmole) dissolved in 4 mL of H₂O. The resulting solution mixture was heated at reflux for 14
10 hrs, and allowed to cool to room temperature. The solution was diluted with ethyl acetate (200mL), and the separated organic phase was dried over MgSO₄. The solution was concentrated and chromatographed on silica gel eluting with 10% ethyl acetate-hexanes to yield the biaryl (157) (2.16 g, 79%) as a colorless oil; ¹H NMR (500 MHz, CDCl₃) (157) δ
15 7.68 (d, 1H), 7.44 (d, 2H), 7.42-7.38 (m, 1H), 6.94 (d, 1H), 4.24 (s, 4H), 3.68 (t, 4H), 3.37 (q, 4H), 3.10 (t, 2H), 2.83 (t, 2H), 2.49 (t, 4H), 1.12 (t, 6H).

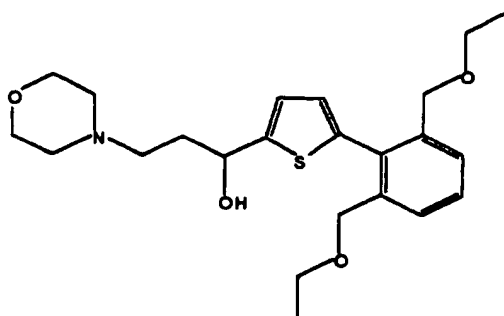


157

20 To a suspension of lithium aluminum hydride (170mg, 4.48mmole) in anhydrous THF (20mL) was added dropwise a solution of the ketone (157) (1.82g, 4.36mmole) in anhydrous THF (10mL) at -40°C, and the mixture stirred for 20 min. The

-56-

reaction mixture was quenched by addition of Rochelle salt aqueous solution (5mL), and stirred overnight at room temperature. The solution was extracted with ethyl acetate (50ml X 2), and the extracts were dried over MgSO_4 , concentrated and chromatographed on silica gel eluting with 2.5% $\text{MeOH-CH}_2\text{Cl}_2$ to yield the alcohol (158) (1.54 g, 84%) as a colorless oil; $^1\text{H NMR}$ (500 MHz, CDCl_3) (158) δ 7.44(d, 2H), 7.38(d, 1H), 6.88(d, 1H), 6.72(d, 1H), 5.16(t, 1H), 4.29(s, 4H), 3.70(t, 4H), 3.38(q, 4H), 2.68(t, 2H), 2.47(brs, 1H), 1.98(dt, 2H), 1.16(t, 6H).

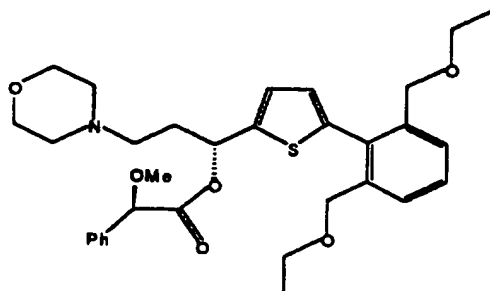


158

To a solution of the alcohol (158) in CH_2Cl_2 (10mL) was added (R)-(-)- α -methoxyphenylacetic acid (1.22g, 7.36mmole), N-ethyl-N'-1-(3-dimethylaminopropyl)-carbodiimide hydrochloride (1.41g, 7.36mmole), and 4-dimethylaminopyridine (3mg, 0.025mmole). The resulting mixture was stirred at room temperature for 2 hrs, and quenched by adding H_2O (5mL). The mixture was diluted with CH_2Cl_2 (20 mL). The organic phase was dried over MgSO_4 , and the solution concentrated and chromatographed on silica gel eluting with diethyl ether to provide the diastereomeric mixture (1.51g, 72%) of the esters as colorless oils. The ester (159) was isolated (290mg, 28%) in pure form through further silica gel chromatography, eluting with diethyl ether; TLC R_f =0.24 (diethyl ether) (Compound 159), R_f =0.31 (diethyl ether) (The diastereomer of

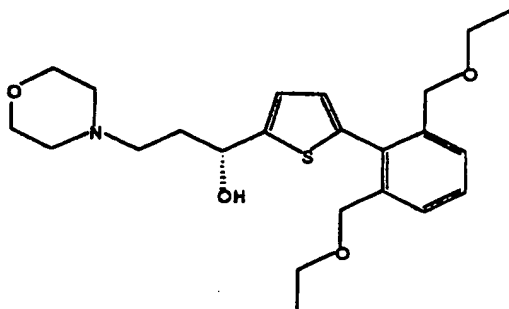
-57-

compound 159); ^1H NMR (500 MHz, CDCl_3) (159) δ 7.46-7.18(m, 8H), 6.83(t, 1H), 6.62(d, 1H), 6.18(t, 1H), 4.77(s, 1H), 4.17(s, 4H), 3.65(m, 4H), 3.40-3.23(m, 7H), 2.40-1.98(m, 8H), 1.13(t, 6H).



159

To a solution of the ester (159) (290mg, 0.511mmole) in methanol (1 mL) was added 1N NaOH solution (4mL, 4 mmole) at room temperature and the mixture stirred for 1hr. The solution was concentrated and extracted with CH_2Cl_2 (10 ml X 2). The extracts were dried over MgSO_4 , concentrated and chromatographed on silica gel eluting with 5% of $\text{MeOH}-\text{CH}_2\text{Cl}_2$ to yield the alcohol (160) (210 mg, 98%) as a colorless oil; ^1H NMR (500 MHz, CDCl_3) (160) δ 7.44(d, 2H), 7.38(d, 1H), 6.88(d, 1H), 6.72(d, 1H), 5.16(t, 1H), 4.29(s, 4H), 3.70(t, 4H), 3.38(q, 4H), 2.68(t, 2H), 2.50(brs, 1H), 1.98(dt, 2H), 1.16(t, 6H).

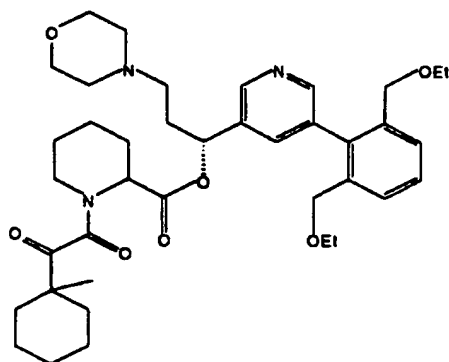


160

-58-

To a solution of the alcohol (160) (20mg, 0.048mmole) in CH_2Cl_2 (2mL) was added the acid (151) (35mg, 0.124 mmole) and 1,3-dicyclohexylcarbodiimide (25mg, 0.121mmole), (-)-camphorsulfonic acid (8.0mg, 0.0345mmole) and 4-dimethylaminopyridine (4.0 mg, 0.0328mmole). The resulting mixture was stirred at room temperature for 6 days, and quenched by adding H_2O (1mL). The aqueous layer was extracted with CH_2Cl_2 (5 ml X 2). The combined organics were dried over MgSO_4 and concentrated. The obtained oil was chromatographed on silica gel eluting with 1% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ to 2% $\text{MeOH}-\text{CH}_2\text{Cl}_2$. The fractions containing the ester (79) were collected and concentrated and the resulting oil was chromatographed on silica gel eluting with 30% ethyl acetate-hexanes to 50% of ethyl acetate-hexanes to yield the ester (79) (11mg, 34%) as a colorless solid; TLC $R_f=0.30$ (5% $\text{CH}_2\text{Cl}_2/\text{MeOH}$); ^1H NMR (500 MHz, CDCl_3) δ 7.50 (d, 2H), 7.40 (dd, 1H), 7.07 (d, 1H), 6.75 (d, 1H), 6.22 (dd, 1H), 5.20 (m, 1H), 4.25 (s, 4H), 3.72 (m, 4H), 3.38 (m, 4H), 3.10 and 2.88 (dt, 1H), 2.50-1.20 (m, 25H), 1.28 (s, 3H), 1.15 (t, 6H).

20

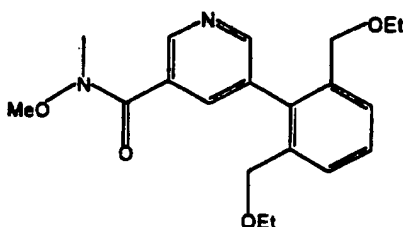
Example 3 -- Preparation of Compound 78

78

To a solution of 138 (6.17 g, 25.2 mmol) and tetrakis(triphenylphosphine)-palladium(0) (0.55 g, 0.48 mmol)

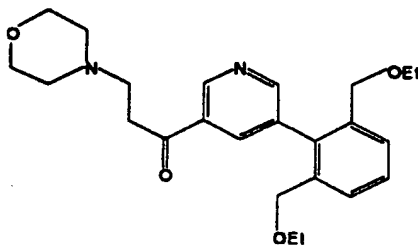
-59-

in toluene (300 mL) was added 154 (5.00 g, 21.0 mmol) in ethanol (20 mL) and sodium carbonate monohydrate (5.20 g, 42 mmol) in water (20 mL). The solution was heated at reflux for 16 hours and the layers separated. The aqueous layer was
5 extracted with ethyl acetate, and the combined organics dried (MgSO_4) and concentrated to an oil. Purification by flash chromatography (1:1 ethyl acetate:hexane as eluent) yielded 6.00 g (80 %) of 152 as a colorless oil.



152'

10 To a solution of 152' (485 mg, 1.35 mmol) in THF (5 mL) at 0°C was added vinyl grignard (6.8 ml of 1.0 M solution in THF, 6.8 mmol) and the reaction stirred at 0°C for 1 hour. Morpholine (235 mg, 2.70 mmol) was added, and the reaction
15 quenched with water. The reaction mixture was extracted with ethyl ether and the combined organics dried (MgSO_4) and concentrated to an oil. Purification by flash chromatography, eluting with 0 to 5% ethanol in ethyl ether, yielded 245 mg (45%) of 161 as a pale yellow oil.

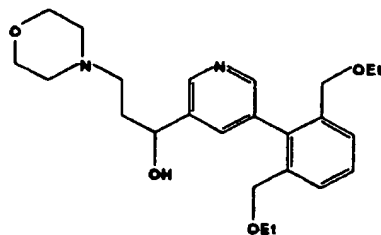


161

-60-

To a solution of 161 (240 mg, 0.58 mmol) in THF at -40°C was added lithium aluminum hydride (23 mg, 0.61 mmol). The reaction was stirred for 20 minutes, quenched with Rochelle's salt and extracted with ethyl acetate.

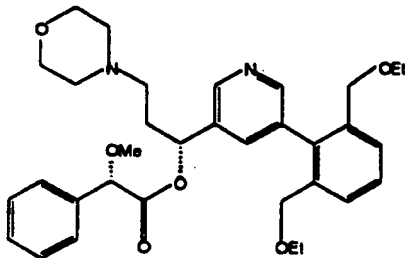
- 5 Combined organics were dried (MgSO₄) and concentrated to an oil. Purification by flash chromatography (2 to 3% methanol in methylene chloride as eluent) yielded



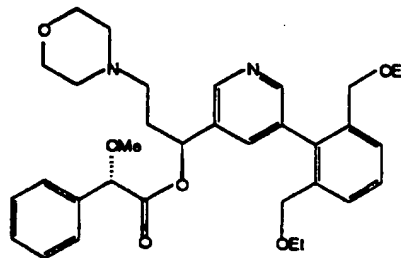
162

- 10 compound 162 (170 mg, 71%) as a colorless oil.

- A solution of 162 (170 mg, 0.41 mmol), S-(+)- α -methoxyphenylacetic acid (206 mg, 1.24 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (238 mg, 1.24 mmol) and catalytic DMAP were combined in dry methylene chloride (5 mL) and stirred for 3 days. The reaction mixture was diluted with methylene chloride and washed with water and saturated bicarbonate solution. The methylene chloride layer was dried (MgSO₄) and concentrated to a yellow oil. Purification by flash chromatography (2% ethanol in ethyl ether as eluent)
- 15 yielded 45 mg (20%) of the less polar diastereomer 163 and 29 mg (13%) of the more polar diastereomer 164 as colorless oils.



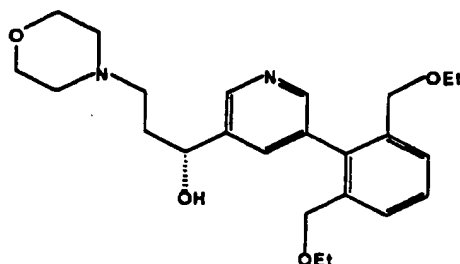
163



164

-61-

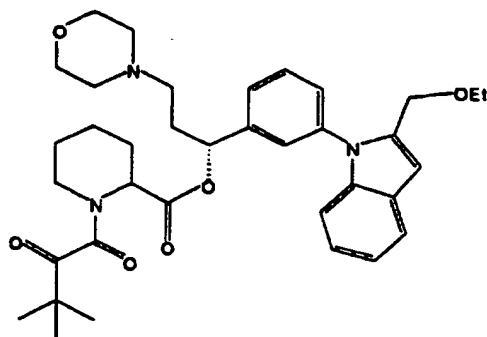
To a solution of 163 (42 mg, 0.075 mmol) in methanol (2 mL) at 0°C was added 1N NaOH (112 uL, 0.112 mmol) and the reaction mixture stirred for 1.5 hours. The mixture was diluted with water and extracted with methylene chloride. Combined organics were dried (MgSO₄) and concentrated to yield 165 (30 mg, 97%) as a colorless oil.



165

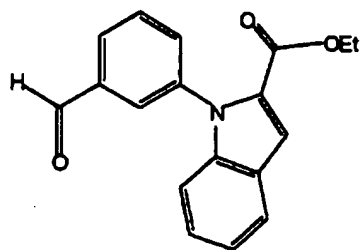
Compound 165 (23 mg, 0.055 mmol), 151 (24 mg, 0.0825 mmol), 1,3-dicyclohexylcarbodiimide (19 mg, 0.0935 mmol) and catalytic camphorsulfonic acid and DMAP were combined in dry methylene chloride (1 mL) and the solution stirred overnight. The methylene chloride was removed in vacuo and the resulting oil purified by chromatography, eluting with 1 to 5% ethanol in methylene chloride, to yield 78 (24 mg, 65%) as a colorless oil and mixture of rotomers. ¹H-NMR (500 MHz, CDCl₃): 1.10 (dt, 6H), 1.24 and 1.15 (s, 1H) 1.36 (m, 8H), 1.93 (m, 4H), 2.3 (m, 8H), 3.13 and 2.88 (dt, 1H), 3.29 (m, 4H), 3.39 and 4.43 (br d, 1H), 3.67 (dd, 4H) 4.08 (dd, 4H) 5.25 and 4.20 (br d, 1H), 5.98 (t, 1H), 7.42 (m, 3H) 7.59 (s, 1H), 8.46 (d, 1H) 8.67 and 8.62 (d, 1H).

-62-

Example 4 -- Preparation of Compound 82

82

To a stirred solution of 21.45g (113 mmole) of ethylindole-3-carboxylate and 66ml (567 mmole) of 3-bromobenzaldehyde in DMF (35ml) was added 81g (567mmole) of copper(I)oxide and the suspension heated at 120°C overnight. The reaction mixture was filtered through celite and partitioned with water and ethyl acetate. The organic phase was washed two times with aqueous potassium bisulfate, two times with aqueous sodium bicarbonate, two times with water, once with brine and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel, using 3:1 methylene chloride/hexane as eluent to give 4.21g of 166.



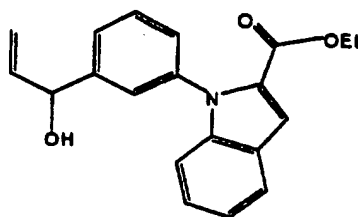
15

166

To a stirred solution of 3.0g (10.2 mmole) of 166 at -78°C in THF (30 ml) was added 25.6ml (25.6 mmole) of 1M vinylmagnesium bromide in THF dropwise. The mixture was allowed to warm to -30°C and stir for 1/2 hour. Reaction

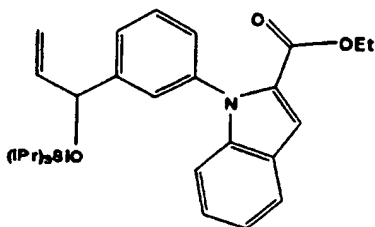
-63-

was quenched with aqueous ammonium chloride and extracted with ethyl acetate. The organic phase was washed once with brine and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue was
5 purified by chromatography on silica gel, using 7:3 hexane/diethyl ether to give 1.80g of 167.



167

To a stirred solution of 1.2g (3.7 mmole) of 167 and 0.5ml (4.8 mmole) of 2,6-lutidine in methylene chloride
10 at 0°C was added 1.2ml (4.4 mmole) of triisopropylsilyltrifluoromethane sulfonate dropwise. The mixture was stirred for twenty minutes at 0°C. The reaction was diluted with methylene chloride and washed two times with aqueous potassium bisulfate, two times with water, once with
15 brine and dried over magnesium sulfate. The solvent was evaporated under reduced pressure to give 1.7g of 168.

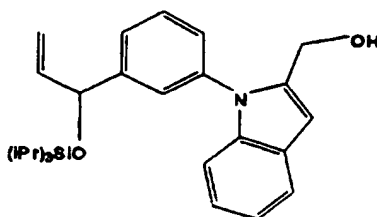


168

To a stirred mixture of 170mg (4.5 mmole) of lithium aluminum hydride in THF (5ml) at 0°C was added 1.7g
20 (3.6 mmole) of 168 in THF (3ml). The mixture was stirred for fifteen minutes and was slowly quenched with aqueous sodium sulfate, then warmed to room temperature and treated with

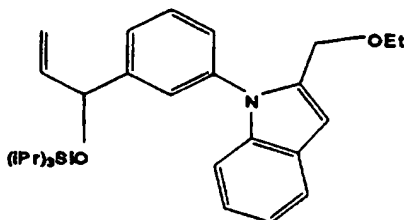
-64-

excess of sodium sulfate powder. The mixture was stirred for fifteen minutes and filtered. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel using 95:5 hexane/ethyl acetate to give 0.86g of 169.



169

To a stirred mixture of 62mg (2.6 mmole) of sodium hydride in THF (6ml) at 0°C was added 0.86g (2.0 mmole) of 169. The mixture stirred for twenty minutes and then treated with 0.4ml (5.0 mmole) of iodoethane, allowed to warm to room temperature and stir overnight. The reaction was recooled to 0°C and slowly quenched with water. Partitioned with water and ethyl acetate. The organic phase was washed two times with water, once with brine, and dried over magnesium sulfate. The solvent was evaporated under reduced pressure to give 0.92g of 170.

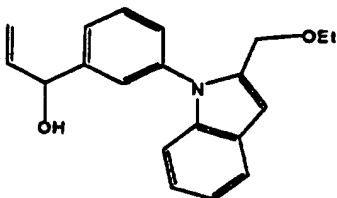


170

To a stirred solution of 0.92g (2.0 mmole) of 170 in THF (5ml) at 0°C was added 2.4mL (2.4 mmole) of 1M tetrabutylammonium fluoride in THF. The reaction was allowed

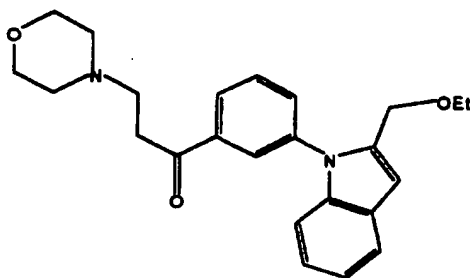
-65-

to warm to room temperature and stir for two hours.
Partitioned with ethyl acetate and dilute hydrochloric acid.
The organic phase was then washed two times with dilute
hydrochloric acid, once with brine, and dried over magnesium
5 sulfate. The residue was purified by chromatography on
silica gel using 3:1 hexane:diethyl ether as eluent to give
470mg of 171.



171

To a stirred mixture of 450mg (1.5 mmole) of 171,
10 1.3g of 4Å powdered sieves, and 0.25mL (2.9 mmole) of
morpholine in methylene chloride (15mL) was added 1.3g (15
mmole) of manganese(IV)oxide. The mixture was allowed to
stir for two hours at room temperature. The reaction was
filtered through celite and evaporated under reduced pressure
15 to give 448mg of 172.

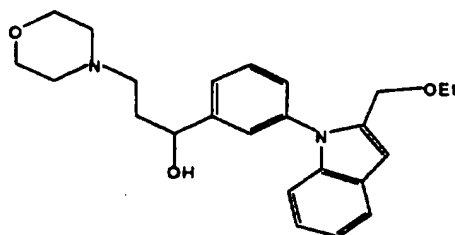


172

To a stirred mixture of 43mg (1.14 mmole) of
lithium aluminum hydride in THF (4ml) at -78°C was added
448mg (1.14 mmole) of 172 dissolved in THF (4ml). The
20 mixture was allowed to stir for two hours and was slowly
quenched with aqueous sodium sulfate, then warmed to room

-66-

temperature and and treated with excess of sodium sulfate powder. The mixture was stirred for fifteen minutes and filtered. The solvent was evaporated under reduced pressure to give 450 mg of 173.



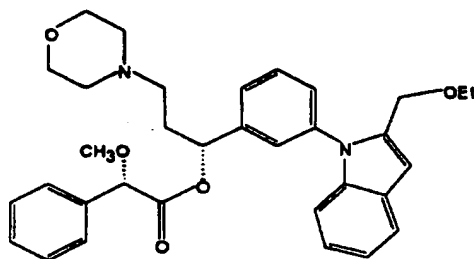
5

173

To a stirred solution of 450mg (1.15 mmole) of 173, 565mg (3.40 mmole) of (+)-(s)- α -methoxyphenylacetic acid, and catalytic 4-dimethylaminopyridine in methylene chloride (7ml) was added 650mg (3.4 mmole) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The reaction was allowed to stir at room temperature overnight, diluted with methylene chloride and washed two times with aqueous sodium bicarbonate, two times with water, once with brine, and dried over sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel using diethyl ether as eluent to give 260mg of the desired higher Rf diastereomer, 174.

10

15



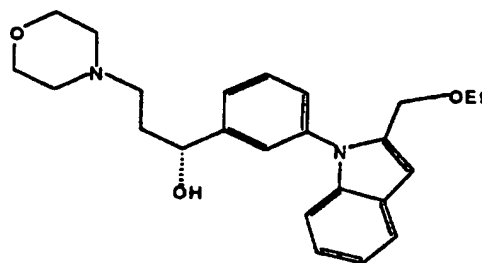
174

To a stirred mixture of 18mg (0.48 mmole) of lithium aluminum hydride in diethyl ether (1ml) at -78°C was

20

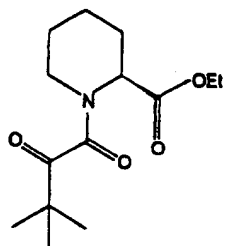
-67-

added 174 dissolved in diethyl ether (1ml). The mixture was allowed to stir for two minutes and was quenched with aqueous sodium sulfate, then warmed to room temperature and treated with excess of sodium sulfate powder. The mixture was
5 stirred for fifteen minutes and filtered. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel using 3:97 ethyl alcohol/ethyl acetate as eluent to give 154mg of 175.



175

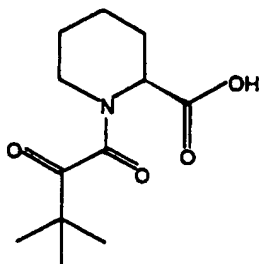
10 To a stirred solution of 250mg (1.02 mmole) of 147 in methylene chloride at 0°C was added 3.08ml (3.08 mmole) of 1M tert-butylmagnesium chloride in THF. The reaction was allowed to stir at 0°C for fifteen minutes and quenched with aqueous ammonium chloride. The aqueous phase was extracted
15 three times with methylene chloride. The organic layers were combined and washed once with brine, dried over magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica using 9:1 hexane/ethyl acetate as eluent to give 102mg
20 of 176.



176

-68-

To a stirred solution of 102mg (0.38 mmole) of 176 in THF (1mL) at 0°C was added 0.76ml (0.76mmole) of 1N lithium hydroxide. The reaction was allowed to warm to room temperature and stir for four hours, then acidified with 10% aqueous potassium bisulfate, and extracted three times with methylene chloride. The organic layers were combined and washed once with brine, dried over magnesium sulfate, and the solvent was evaporated under reduced pressure to give 85 mg of 177.



10

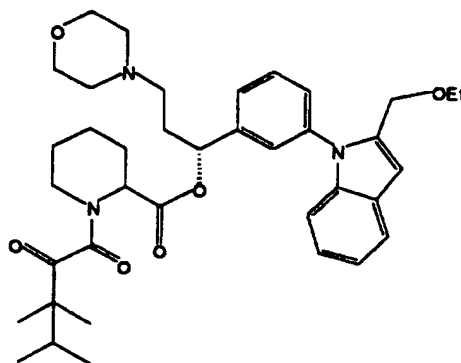
177

To a stirred solution of 20mg (0.051 mmole) of 175, 18mg (0.076 mmole) of 177, 18mg (0.086mmole) of dicyclohexyl carbodiimide, and catalytic camphorsulfonic acid in methylene chloride (1ml) at room temperature was added catalytic N,N-dimethylaminopyridine. The reaction was allowed to stir overnight. The solvent was evaporated under reduced pressure and purified by chromatography on silica using 3:2 ethyl acetate/hexane to give 23mg of 82. TLC Rf: 0.12 (3:2 ethyl acetate/hexane), ¹HNMR (500 MHz, CDCl₃): δ 1.14 (s, 3H), 1.23 (s, 9H), 1.25-1.38 (m, 1H), 1.40-1.56 (m, 1H), 1.57-1.80 (m, 3H), 2.0 (m, 1H), 2.20 (m, 1H), 2.30-2.47 (m, 6H), 3.17 (m, 1H), 3.29 (m, 2H), 3.44 (q, 2H), 3.69 (m, 4H), 4.45 (m, 2H), 5.29 (m, 1H), 5.97 (t, 1H), 6.67 (s, 1H), 7.15 (m, 3H), 7.44 (m, 3H), 7.52 (d, 1H), 7.64 (d, 1H).

-69-

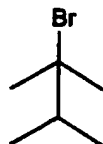
Example 5 -- Preparation of Compound 94

To a solution of 16g (157 mmole) of 2,3-



94

5 dimethyl-2-butanol and 20.4g (235 mmole) of lithium bromide at 0°C was added 36.4ml (314 mmole) 48% aqueous hydrobromic acid dropwise. The reaction was allowed to stir for two hours and then warmed to room temperature. The organic phase was washed three times with ethylene glycol dried over sodium sulfate to give 20g of 176.

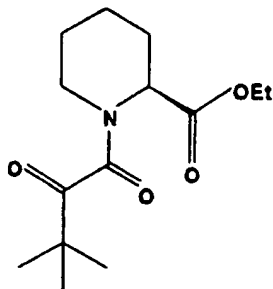


176

10 To a solution of 5g (30.3 mmole) of 176 in THF (5ml) was added 810mg (33.3 mmole) of magnesium turnings. After initial exotherm, the reaction was heated to reflux for one hour. The solution was cooled to room temperature and
15 added to 900mg (3.7 mmole) of 147 in methylene chloride (5ml) and allowed to stir overnight. The reaction was cooled to 0°C and acidified with 0.5N aqueous hydrochloric acid, washed organic phase two times with water, once with aqueous sodium bicarbonate, two times with water, once with brine, and dried
20 over sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by

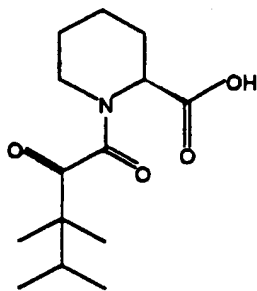
-70-

chromatography on silica using 9:1 hexane/ethyl acetate to give 121mg of 177.



177

To a solution of 121mg (0.41 mmole) of 177 in THF
5 (1ml) at 0°C was added 1.0 ml (1.04 mmole) of 1N aqueous
lithium hydroxide dropwise. The reaction was allowed to warm
to room temperature, stirred overnight, re-cooled to 0°C,
acidified with 1.1ml 1N hydrochloric acid, and extracted
three times with benzene. The organic phase was cooled to
10 0°C, subjected to a stream of nitrogen for 0.5 hours and then
dried over sodium sulfate. The solvent was evaporated under
reduced pressure to give 95 mg of 178.



178

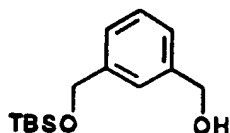
To a stirred solution of 13mg (0.033mmole) of 175,
15 14mg (0.052 mmole) of 178, 12mg (0.056 mmole) of dicyclohexyl
carbodiimide, and catalytic camphorsulfonic acid in methylene
chloride (1ml) at room temperature was added catalytic N,N-
dimethylaminopyridine. The reaction was allowed to stir

-71-

overnight. The solvent was evaporated under reduced pressure and the product purified by chromatography on silica gel using 99:1 methylene chloride/ethyl alcohol to give 10mg of 94. TLC Rf: 0.41 (95:5 diethyl ether/ethyl alcohol), ¹HNMR (500 MHz, CDCl₃): δ 0.87 (d, 6H), 1.13 (s, 6H), 1.28 (s, 3H), 1.29-1.82 (m, 6H), 2.02 (m, 1H), 2.15-2.30 (m, 2H), 2.32-2.48 (m, 6H), 3.14 (m, 1H), 3.38 (m, 1H), 3.44 (q, 2H), 3.71 (m, 4H), 4.45 (s, 2H), 5.3 (d, 1H), 5.97 (t, 1H), 6.68 (s, 1H), 7.13 (m, 3H), 7.45 (m, 3H), 7.54 (d, 1H), 7.65 (d, 1H).

10 Example 6 -- Preparation of Compound 27

To a solution of 31.05 g (225 mmol) of 1,3-benzenedimethanol (Aldrich Chemical Co.) in 500 mL of dry THF was added 7.76 g (232 mmol) of 80% sodium hydride. To this suspension was added 34.97 g (232 mmol) of tert-butyltrimethylsilyl chloride and the resulting mixture was allowed to stir overnight at room temperature. The reaction mixture was then quenched with water, extracted into ethyl acetate, dried over MgSO₄ and concentrated. Flash chromatography (elution with 5:1 hexane/ethyl acetate) gave 33.3 g of the alcohol (179) (59%).

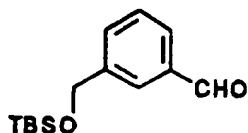


179

To a solution of 33.2 g (131.5 mmol) of the alcohol (179) in 100 mL of CH₂Cl₂ at 0°C was added 350 mg (2.2 mmol) of TEMPO, 295 mL (197 mmol) of 0.67 M sodium hypochlorite containing 7.5 g of sodium bicarbonate and 1.34 g (13.1 mmol) of sodium bromide. The resulting mixture was allowed to stir at 0°C for 0.5 h and then extracted into ethyl acetate. The

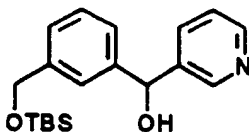
-72-

organic phase was washed sequentially with aqueous solutions of potassium iodide and sodium thiosulfate and then dried over MgSO_4 and concentrated. Flash chromatography (elution with 20% ethyl acetate in hexane) gave 31.4 g (95%) of the
5 aldehyde 180.



180

To a solution of 8.1 g (51.1 mmol) of 3-bromopyridine in 100 mL of dry ether at -78°C was added 31 mL of a 1.6 M hexane solution of $n\text{-BuLi}$ (31 mL) and the resulting mixture was allowed to stir at -78°C for 20 min.
10 To this solution was added a solution of 12.8 g (51.1 mmol) of aldehyde 180 in 190 mL of dry ether and this solution was then allowed to stir at -78°C for 1h. The reaction mixture was poured into saturated aqueous NH_4Cl , the layers were separated and the aqueous layer was extracted with ether. The
15 organic layers were combined, dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The obtained residue was chromatographed on silica gel with hexane/ethyl acetate (5:3) to give 12 g (71%) of alcohol 181 as a colorless oil. NMR (500 MHz, CDCl_3) 0.06 (6H,s) 0.91 (9H,s) 4.71 (2H,s) 5.83
20 (1H,s) 7.68 (6H,m) 8.40 (1H,m) 8.52 (1H,d, $J=2.3\text{Hz}$).

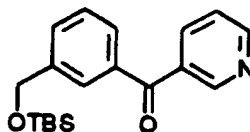


181

A mixture of 12 g (36.4 mmol) of alcohol 181, 21 g (241.5 mmol) of MnO_2 and 6.8 g of 4Å molecular sieves in 90 mL of CH_2Cl_2 was allowed to stir overnight at room temperature. The reaction mixture was filtered through

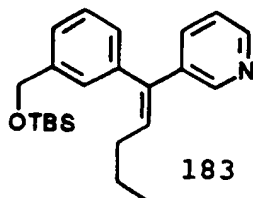
-73-

celite and concentrated under reduced pressure to give 10.8 g (90%) of ketone 182 as a colorless oil. NMR (500 MHz, CDCl₃) 0.06 (6H, s) 0.89 (9H, s) 4.76 (2H, s) 7.3-7.7 (6H, m) 8.77 (1H, d, J=1.7Hz) 8.95 (1H, d, J=1.7Hz).



182

5 To a suspension of 8.1 g (20.28 mmol) of *n*-butyltriphenylphosphonium bromide in 120 mL of dry THF was added 11.5 mL of a 1.6 M hexane solution of *n*-BuLi at 0°C and the resulting red solution was stirred at 0°C for 30 min. To this solution was added 4.0 g (12.21 mmol) of ketone 182 in
10 10 mL of dry THF at 0°C and this mixture was warmed to room temperature over a period of an hour. Flash chromatography (elution with 3:1 hexane:ethyl acetate) gave a mixture of the *E*-olefin 183 and the corresponding *Z*-olefin isomer. This
15 mixture was further chromatographed on silica gel with hexane/ethyl acetate (4:1) to give pure olefin 183 (970 mg, 19%) as a colorless oil. NMR (500 MHz, CDCl₃) 0.08 (6H, s) 0.91 (12H, m) 1.4-1.6 (2H, m) 2.1-2.2 (2H, m) 4.75 (2H, s) 6.11 (1H, t, J=7.4Hz) 7.0-7.5 (6H, m) 8.44 (1H, d, J=4.5Hz) 8.54 (1H, d, J=1.1Hz).

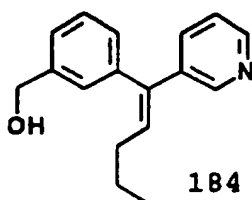


183

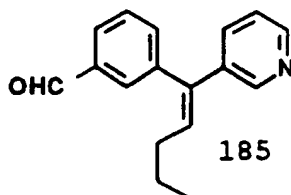
20 A mixture of 960 mg (2.61 mmol) of olefin (183) and 13 mL of a 1 M solution of tetrabutylammonium fluoride in THF was stirred for 30 min at room temperature. This mixture was then poured into water and extracted with ethyl acetate. The

-74-

organic layer was dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane/ethyl acetate (5:1) to give alcohol 184 (700 mg, 87%) as a colorless oil. NMR (500 MHz, CDCl_3) 0.88 (3H, m) 1.37 (2H, m) 2.05 (2H, m) 4.69 (2H, s) 6.12 (1H, t, $J=7.4\text{Hz}$) 7.0-7.5 (6H, m) 8.33 (1H, m) 8.46 (1H, d, $J=2.2\text{Hz}$).



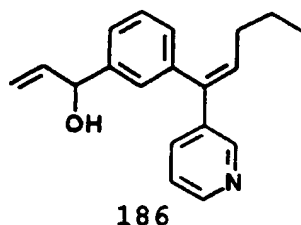
A mixture of 579 mg (2.24 mmol) of alcohol 184, 1.2 g (13.8 mmol) of MnO_2 and 1.0 g of 4 Å molecular sieves in 10 mL of CH_2Cl_2 was stirred overnight at room temperature. This mixture was then filtered and the filtrate was concentrated under reduced pressure to give aldehyde (185) (560 mg, 99%) as a colorless oil. NMR (500 MHz, CDCl_3) 0.91 (3H, m) 1.50 (2H, m) 2.09 (2H, m) 6.22 (1H, t, $J=7.4\text{Hz}$) 7.1-7.9 (6H, m) 8.4-8.6 (2H, m) 10.03 (1H, s).



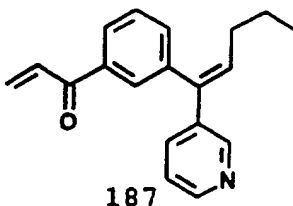
To a solution of 560 mg (2.23 mmol) of aldehyde 185 in 10 mL of dry THF at -10°C was added 4.5 mL of a 1 M THF solution of vinyl magnesium bromide dropwise and the resulting mixture was stirred at -10°C for 30 min. The mixture was then poured into saturated aqueous NH_4Cl and extracted with CHCl_3 . The organic layer was dried over Na_2SO_4 ,

-75-

filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel with hexane/ethyl acetate (1:1) to give allyl alcohol 186 (310 mg, 50%) as a colorless oil. NMR (500 MHz, CDCl₃) 0.89 (3H, t, J=7.3Hz) 1.46 (2H, m) 2.10 (2H, m) 5.1-5.3 (3H, m) 5.9-6.2 (2H, m) 7.0-7.2 (3H, m) 7.3-7.5 (3H, m) 8.33 (1H, m) 8.44 (1H, d, J=2.4Hz).



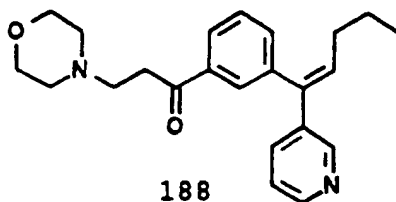
A mixture of 310 mg (1.12 mmol) of allyl alcohol 186, 488 mg (5.61 mmol) of MnO₂ and 500 mg of 4 Å molecular sieves was stirred overnight at room temperature. The reaction mixture was then filtered and the filtrate was concentrated under reduced pressure to give enone 187 (244 mg, 79%) as a colorless oil. NMR (500 MHz, CDCl₃) 0.92 (3H, t, J=7.3Hz) 1.50 (2H, m) 2.11 (2H, m) 5.93 (1H, m) 6.22 (1H, t, J=7.4Hz) 6.44 (1H, m) 7.1-7.3 (2H, m) 7.3-7.6 (3H, m) 7.76 (1H, s) 7.92 (1H, m) 8.4-8.6 (2H, m).



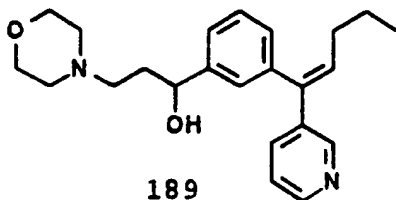
A mixture of 244 mg (0.88 mmol) enone 187, 153 mg (1.75 mmol) of morpholine (153 mg) and 1.83 mL (13.13 mmol) of triethylamine in 5.0 mL of CH₃CN was stirred for 30 min at room temperature and the mixture was then concentrated under reduced pressure. The residue was chromatographed on silica gel with CHCl₃/MeOH (100:3) to give ketone (188) (240mg, 75%).

-76-

as a colorless oil. NMR (500MHz, CDCl₃) 0.91 (3H, t, J=7.3Hz) 1.4-1.6 (2H, m) 2.0-2.2 (2H, m) 2.44 (4H, m) 2.82 (2H, t, J=6.8Hz) 3.17 (2H, t, J=6.8Hz) 3.70 (4H, m) 6.19 (1H, t, J=7.3Hz) 7.1-7.6 (4H, m) 7.76 (1H, m) 7.91 (1H, m) 8.49 (2H, m).



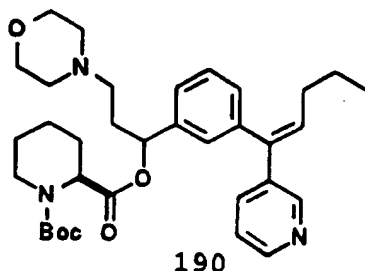
- 5 To a solution of 240 mg (0.67 mmol) of ketone (188) in 5.0 mL of MeOH at 0°C was added 25 mg (0.67 mmol) of NaBH₄ and the resulting mixture was stirred at 0°C for 30 min. The reaction mixture was then poured into saturated aqueous NH₄Cl and extracted with CHCl₃. The organic layer was dried over
- 10 Na₂SO₄, filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel with CHCl₃/MeOH (100:3) to give alcohol (189) (208 mg, 85%) as a colorless oil. NMR (500 MHz, CDCl₃) 0.94 (3H, t, J=7.3Hz) 1.4-1.6 (2H, m) 1.8-2.0 (2H, m) 2.0-2.2 (2H, m) 2.4-2.7 (6H, m) 3.71 (4H, m) 4.93
- 15 (1H, m) 6.11 (1H, t, J=7.3Hz) 7.0-7.2 (2H, m) 7.3-7.5 (4H, m) 8.41 (1H, m) 8.50 (1H, d, J=1.5Hz).



- To a solution of 141 mg (0.61 mmol) of Boc-L-pipecolic acid in 2.0 mL of CH₂Cl₂ and 2.0 mL of DMF was added 205 mg (0.56 mmol) of alcohol (189), 119 mg (0.62 mmol) of
- 20 EDC and a catalytic amount of DMAP. The mixture was stirred overnight at room temperature and was then poured into water. The layers were separated and the organic layer was dried

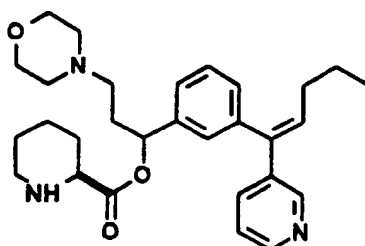
-77-

over Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was chromatographed on silica gel with $\text{CHCl}_3/\text{MeOH}$ (100:1) to give ester (190) (207 mg, 64%) as a colorless oil. NMR (500 MHz, CDCl_3) 0.91 (3H, t, $J=7.3\text{Hz}$) 1.2-1.8 (15H, m) 1.8-2.0 (1H, m) 2.0-2.3 (4H, m) 2.3-2.5 (6H, m) 2.7-2.9 (2H, m) 3.67 (4H, m) 3.7-4.1 (1H, m) 4.7-4.9 (1H, m) 5.90 (1H, m) 6.14 (1H, t, $J=7.3\text{Hz}$) 7.1-7.2 (3H, m) 7.3-7.5 (3H, m) 8.4-8.5 (2H, m).



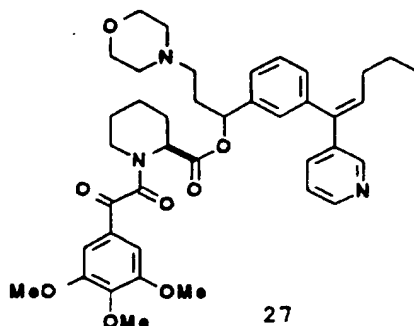
To a solution of 207 mg (0.36 mmol) of ester (190) in 1.5 mL of CH_2Cl_2 at room temperature was added 3.2 mL of trifluoroacetic acid and the mixture was stirred for 1 h at room temperature. The reaction mixture was then neutralized by the dropwise addition of saturated aqueous K_2CO_3 . The layers were separated and the organic layer was dried over Na_2SO_4 , filtered and concentrated to give amine (191) (155 mg, 89%) as a pale brown oil. NMR (500 MHz, CDCl_3) 0.95 (3H, t, $J=7.3\text{Hz}$) 1.3-1.7 (6H, m) 1.7-2.1 (2H, m) 2.1-2.3 (3H, m) 2.3-2.5 (8H, m) 2.5-2.7 (1H, m) 3.0-3.1 (1H, m) 3.3-3.5 (1H, m) 3.67 (4H, m) 5.89 (1H, m) 6.13 (1H, t, $J=7.3\text{Hz}$) 7.0-7.2 (3H, m) 7.3-7.5 (3H, m) 8.44 (2H, m).

-78-



191

To a solution of 155 mg (0.32 mmol) of amine (191) in 2.8 mL of CH_2Cl_2 at room temperature was added 80 mg (0.33 mmol) of 3,4,5-trimethoxybenzoylformic acid and 92 mg (0.48 mmol) of EDC. After stirring overnight at room temperature, the reaction mixture was partitioned with water and the organic layer was dried over Na_2SO_4 , filtered and concentrated. The residue was chromatographed on silica gel with $\text{CHCl}_3/\text{MeOH}$ (100:5) to give the diastereomeric amides (27) (166 mg, 74%) as a mixture of rotamers. NMR (500 MHz, CDCl_3) 1.8-2.0 (3H, m) 1.4-1.6 (6H, m) 1.6-1.9 (2H, m) 2.0-2.2 (3H, m) 2.2-2.5 (7H, m) 3.0-3.3 (1H, m) 3.3-3.5 (1H, m) 3.68 (4H, m) 3.8-4.0 (9H, m) 5.38 (1H, d, $J=3.8\text{Hz}$) 5.90 (1H, m) 6.14 (1H, m) 7.1-7.3 (3H, m) 7.3-7.5 (5H, m) 8.42 (2H, m). The two diastereomeric mixture was further separated by HPLC (ODS coloum with 73: 27 MeOH - H_2O) to give 38 mg and 21 mg of each isomer.



27

Example 7 -- MDR Sensitization Assays

To assay the ability of the compounds according to this invention to increase the antiproliferative activity of a drug, cell lines which are known to be resistant to a particular drug may be used. These cell lines include, but are not limited to, the L1210, P388D, CHO and MCF7 cell lines. Alternatively, resistant cell lines may be developed. The cell line is exposed to the drug to which it is resistant, or to the test compound; cell viability is then measured and compared to the viability of cells which are exposed to the drug in the presence of the test compound.

To carry out assays using L1210 mouse leukemia cells transformed with the pHaMDR1/A retrovirus carrying a MDR1 cDNA, we follow the procedure described by Pastan et al., Proc. Natl. Acad. Sci., Vol. 85, 4486-4490 (1988). The resistant line, labelled L1210VMDRC.06, is obtained from Dr. M. M. Gottesman of the National Cancer Institute. Drug-resistant transfectants are selected by culturing cells in 0.06 mg/ml colchicine.

Multi-drug resistance assays are conducted by plating cells (2×10^3 , 1×10^4 , or 5×10^4 cells/well) in 96 well microtiter plates and exposing them to a concentration range of doxorubicin (50 nM-10 μ M) in the presence or absence of multi-drug resistance modifier compounds ("MDR inhibitors") of this invention (1, 2.5 or 10 μ M) as described in Ford et al., Cancer Res., Vol. 50, 1748-1756. (1990). After culture for 3 days, the viability of cells is quantitated using MTT (Mossman) or XTT dyes to assess mitochondrial function. Also see, Mossman T., J. Immunol. Methods, Vol. 65, 55-63 (1983).

Proton nuclear magnetic resonance (^1H NMR) spectra are recorded at 500 MHz on a Bruker AMX 500. Chemical shifts are reported in parts per million (δ) relative to Me_4Si (δ

0.0). Analytical high performance liquid chromatography is performed on either a Waters 600E or a Hewlett Packard 1050 liquid chromatograph.

Results are determined by comparison of the IC₅₀ for doxorubicin alone to the IC₅₀ for doxorubicin+ MDR inhibitor. An MDR ratio is calculated (IC₅₀ Dox/ IC₅₀ Dox + Inhibitor) and the integer value used for comparison of compound potencies.

Compounds according to this invention were tested for intrinsic antiproliferative or cytotoxic activity. The results are summarized in Table 2 below.

TABLE 2

| | Compound # | Date | IC50 DXR Alone (nM) | IC50 (DXR + 2.5 µM VA) (nM) | MDR Ratio |
|----|------------|---------|---------------------|-----------------------------|-----------|
| 15 | 22 | 5/12/95 | 700 | 550 | 1.3 |
| | 22 | 4/21/95 | 2100 | 170 | 12.4 |
| | 24 | 5/12/95 | 700 | <50 | >14.0 |
| | 26 | 5/12/95 | 700 | 80 | 8.8 |
| | 27 | 5/12/95 | 700 | 55 | 12.7 |
| 20 | 28 | 5/12/95 | 700 | 70 | 10.0 |
| | 30 | 5/12/95 | 700 | 50 | 14.0 |
| | 32 | 5/12/95 | 700 | 60 | 11.7 |
| | 40 | 5/12/95 | 700 | 280 | 2.5 |
| | 43 | 5/12/95 | 700 | 50 | 14.0 |
| 25 | 52 | 5/12/95 | 700 | 170 | 4.1 |
| | 52 | 4/21/95 | 2100 | 360 | 5.8 |
| | 59 | 5/12/95 | 700 | 65 | 10.8 |
| | 61 | 5/12/95 | 700 | 280 | 2.5 |
| | 71 | nd | | | |
| 30 | 76 | 5/12/95 | 700 | 110 | 6.4 |
| | 79 | 5/12/95 | 700 | 90 | 7.8 |
| | 89 | 5/12/95 | 700 | 140 | 5.0 |
| | 90 | 5/12/95 | 700 | 95 | 7.4 |
| | 91 | 5/12/95 | 700 | 260 | 2.7 |
| 35 | 100 | 5/12/95 | 700 | <50 | >14.0 |
| | 101 | nd | | | |
| | 105 | 5/12/95 | 700 | 130 | 5.4 |
| | 107 | 5/12/95 | 700 | 80 | 8.8 |
| | 110 | nd | | | |
| 40 | 111 | 5/12/95 | 700 | 75 | 9.3 |

| | | | | |
|-----|---------|------|-----|------|
| 112 | 5/12/95 | 700 | 65 | 10.8 |
| 113 | 5/12/95 | 700 | 100 | 7.0 |
| 119 | 5/12/95 | 700 | 700 | 1.0 |
| 123 | 4/21/95 | 2100 | 170 | 12.4 |

5 Example 8 -- Inhibition of MRP-Mediated MDR

 In order to demonstrate that the compounds of
this invention are effective in reversing MPR-
mediated MDR, in addition to P-glycoprotein-mediated MDR,
we assay inhibition in a non-P-glycoprotein expressing cell
10 line.

 We plate HL60/ADR cells in 96 well microtiter
plates (4×10^4 cells/well). The cells are then exposed to
various concentrations of doxorubicin (50 nM to 10 μ M) in
the presence or absence of various compounds of this
15 invention at various concentrations (0.5 - 10 μ M). After
culturing the cells for 3 days, their viability is
quantitated using the XTT dye method to assess
mitochondrial function. Results are expressed as a ratio
of the IC_{50} for doxorubicin alone to the the IC_{50} for
20 doxorubicin plus MDR inhibitor. IC_{50} values are expressed
in nM. The results indicate that MRP-mediated MDR is
inhibited by compounds of this invention.

Example 9 -- FKBP12 Binding Assay

 The inhibition of FKBP rotomase activity by the
25 preferred FKBP12 binding compounds present in the
compositions of this invention was assayed. In this assay
various quantities of FKBP12 binding compound (0.1 nM - 10
 μ M) were added to cis-N-succinyl-Ala-Ala-Pro-Phe-p-
nitroanilide in the presence of FKBP12 and chymotrypsin.
30 FKBP12 converts the cis form of the substrate to the trans
form. This allows chymotrypsin to cleave p-nitroanilide
from the substrate. Release of p-nitroanilide was measured
spectrophotometrically. This assay allowed me to measure

the change in the first order rate constant of the rotomase activity as a function of FKBP12 binding compound concentration and yielded an estimate of apparent K_i . The most preferred FKBP12 binding compounds utilized in the
5 compositions and methods of this invention and their calculated K_i are tabulated below.

| | Cmpd # | K _i (nM) | Cmpd # | K _i (nM) | Cmp d # | K _i (nM) | Cmpd # | K _i (nM) |
|----|-----------|------------------------|-----------|------------------------|---------------|------------------------|-----------|------------------------|
| | 3 | 300 | 36 | >30 | 69 | 745 | 102 | 700 |
| | 4 | >5000 | 37 | <1 | 70 | 45 | 103 | 21 |
| 5 | 5 | 4000 | 38 | 70 | 71 | 364 | 104 | 870 |
| | 6 | 24 | 39 | 45 | 72 | 225 | 105 | 94 |
| | 7 | >10000 | 40 | 500 | 73 | 718 | 106 | 2100 |
| | 8 | 1000 | 41 | 67 | 74 | 500 | 107 | 7600 |
| | 9 | 7 | 42 | 2100 | 75 | 188 | 108 | 5100 |
| 10 | 10 | 16 | 43 | 180 | 76 | 400 | 109 | >10000 |
| | 11 | 7 | 44 | 53 | 77 | 2100 | 110 | >30000 |
| | 12 | 30 | 45 | 230 | 78 | 77 | 111 | 800 |
| | 13 | 5 | 46 | 100 | 79 | 12 | 112 | 930 |
| | 14 | 12 | 47 | 84 | 80 | 230 | 113 | 61 |
| 15 | 15 | 1 | 48 | 120 | 81 | 189 | 114 | 172 |
| | 16 | 3300 | 49 | >10000 | 82 | 38 | 115 | 44 |
| | 17 | 0.6 | 50 | >10000 | 83 | 315 | 116 | 130 |
| | 18 | 0.4 | 51 | >10000 | 84 | 2000 | 117 | 150 |
| | 19 | 27 | 52 | 200 | 85 | 1.0 | 118 | 70 |
| 20 | 20 | 5.8 | 53 | 70 | 86 | 14 | 119 | >5000 |
| | 21 | 90 | 54 | >50000 | 87 | 2.3 | 120 | 80 |
| | 22 | 31 | 55 | >10000 | 88 | 64 | 121 | 85 |
| | 23 | 2500 | 56 | >5000 | 89 | 263 | 122 | 32 |
| | 24 | 0.5 | 57 | 930 | 90 | 9 | 123 | 2800 |
| 25 | 25 | 30 | 58 | >9000 | 91 | 8 | 124 | 1000 |
| | 26 | 2.4 | 59 | 71 | 92 | 32 | 125 | 1100 |
| | 27 | 260 | 60 | 57 | 93 | 44 | 126 | 3000 |
| | 28 | 20 | 61 | >10000 | 94 | 16 | 127 | 19 |
| | 29 | 1 | 62 | 100 | 95 | 14 | 128 | 41 |
| 30 | 30 | 27 | 63 | >5000 | 96 | 28 | 129 | 1200 |
| | 31 | 130 | 64 | 340 | 97 | 930 | 130 | 490 |
| | 32 | 9.8 | 65 | 490 | 98 | 62 | 131 | 90 |
| | 33 | 12 | 66 | 55 | 99 | ND | 132 | >10000 |
| | 34 | 6 | 67 | 270 | 100 | 380 | 133 | 315 |
| 35 | 35 | 380 | 68 | >5000 | 101 | 500 | | |

Example 10 -- Assay of NeuriteOutgrowth in PC12 Cultures

The ability of the compounds of this invention to bind to FKBP-12 and inhibit its rotomase activity suggested
5 that these compounds could also stimulate nerve growth.

In order to directly determine the neurotrophic activity of the compounds utilized in this invention, the assay described by W. E. Lyons et al., Proc. Natl. Acad. Sci. USA, 91, pp. 3191-95 (1994) is carried out.

10 PC12 rat pheochromocytoma cells are maintained at 37°C and 5% CO₂ in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated horse serum (HS) and 5% heat-inactivated fetal bovine serum (FBS). The cells are then plated at 10⁵ per 35mm culture well coated
15 with 5 µg/cm² rat tail collagen and allowed to attach. The medium is then replaced with DMEM + 2% HS and 1% FBS, NGF (1-100 ng/ml) and varying concentrations of an FKBP12 binding compound (0.1 nM - 10 µM). Control cultures are administered NGF without FKBP12 binding compound.

20 The compounds of this invention are able to increase neurite outgrowth in these cells over and above the outgrowth caused by NGF alone.

Example 11 -- Assay of Neurite Outgrowth
in Dorsal Root Ganglion Culture

25 Another way to directly determine the neurotrophic activity of the FKBP12 binding compounds utilized in this invention is the dorsal root ganglion culture assay also described by W. E. Lyons et al., Proc. Natl. Acad. Sci. USA, 91, pp. 3191-95 (1994).

30 In this assay, dorsal root ganglia are dissected from 16 day rat embryos and cultured in collagen-coated 35mm dishes with N2 medium at 37°C in a 15% CO₂ environment. Sensory ganglia are then treated with various concentrations of NGF (0 - 100 ng/ml) and an FKBP12 binding

compound (0.1 nM - 10 μ M). Ganglia are observed every two days under a phase contrast microscope and axon lengths are measured. Control cultures either lack FKBP12 binding compound or lack FKBP12 binding compound and NGF.

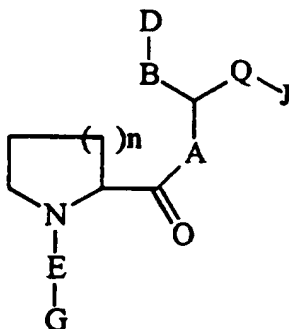
5 The FKBP12 binding compounds utilized in this invention cause an increase in neurite outgrowth over control cultures which lack such compounds in both the presence and absence of NGF.

10 While we have hereinbefore presented a number of embodiments of this invention, it is apparent that our basic construction can be altered to provide other
embodiments which utilize the methods of this invention. Therefore, it will be appreciated that the scope of this
invention is to be defined by the claims appended hereto
15 rather than the specific embodiments which have been presented hereinbefore by way of example.

CLAIMS

We claim:

1. A compound of formula (I):



(I)

wherein:

A is CH₂, O, NH or N-[(C1-C4)-alkyl];

B is (C1-C6)-straight or branched alkyl, or (C2-C6)-straight or branched alkenyl or alkynyl, wherein one of the carbon atoms of B is optionally replaced by O, S, SO, SO₂, NH or N-[(C1-C4)-alkyl];

D is 1-[(C1-C4)-alkyl]-4-piperidinyl; 1-piperazinyl; 1-[(C1-C4)-alkyl]-4-piperazinyl; a 5-7-membered cycloalkyl or cycloalkenyl ring optionally comprising substituents at the 3 and/or 4 position of said ring, wherein said substituents are selected from oxo, OH, (C1-C4)-alkyl, O-[(C1-C4)-alkyl], O-[(C2-C4)-alkenyl], NH₂, N,N di-[(C1-C4)-alkyl]amino or halogen; or a monocyclic or bicyclic aromatic ring structure consisting of 5 to 6 members in each ring and optionally comprising up to 4 heteroatoms independently selected from N, O or S;

E is SO₂ or -C(O)-C(O)-;

G is 1-[(C1-C4)-alkyl]-4-piperidinyl, 1-piperazinyl, 1-[(C1-C4)-alkyl]-4-piperazinyl, (C1-C7)-straight or branched alkyl, (C2-C7)-straight or branched alkenyl or

alkynyl, (C5-C7)-cycloalkyl, or a monocyclic or bicyclic aromatic ring structure consisting of 5 to 6 members in each ring; wherein up to two carbon atoms in any G are optionally replaced independently by O, S, SO, SO₂ or N;

wherein G optionally comprises up to three substituents independently selected from halogen, hydroxyl, (C1-C6)-straight or branched alkyl, (C2-C6)-straight or branched alkenyl, O-[(C1-C5)-straight or branched alkyl], O-[(C2-C5)-straight or branched alkenyl], O-benzyl, amino, carboxyl, N-[(C1-C5)-straight or branched alkyl], N-[(C2-C5)-straight or branched alkenyl], trifluoromethyl or trifluoromethoxy; and wherein one carbon atom of any individual substituent is optionally replaced by O, N or S;

Q is a five membered aromatic ring containing 1 to 2 heteroatoms selected from N, O or S, or a six membered aromatic ring containing 0 to 2 heteroatoms selected from N, O or S;

J is a monocyclic or bicyclic aromatic ring structure attached to the 3 position of Q consisting of 5 to 6 members in each ring, optionally comprising up to four heteroatoms independently selected from O, S, or N; and

wherein J optionally comprises up to 3 substituents independently selected from halo, OH, CH₂OH, NO₂, SO₃H, trifluoromethyl, trifluoromethoxy, O-phenyl, 1,2-methylenedioxy, NR¹R², amino, carboxyl, N-[(C1-C5)-straight or branched alkyl]-carboxamide, N-[(C2-C5)-straight or branched alkenyl]-carboxamide, N-morpholinocarboxamide, N-benzylcarboxamide, N-thiomorpholinocarboxamide, N-picolinoylcarboxamide, morpholinyl, piperidinyl, O-R³, CH₂-(CH₂)_q-R³, O-(CH₂)_q-R³, (CH₂)_q-O-R³, CH=CH-R³, (C1-C6)-straight or branched alkyl, or (C2-C6)-straight or branched alkenyl, wherein in any substituent one carbon atom is optionally replaced by a heteroatom selected from the group consisting of O, S, SO, SO₂, NH or N-[(C1-C4)-alkyl];

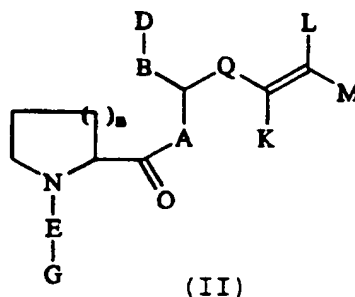
wherein R^1 and R^2 are independently selected from the group consisting of hydrogen, (C1-C6)-straight or branched alkyl, (C2-C6)-straight or branched alkenyl or alkynyl and benzyl;

R^3 is selected from the group consisting of 4-methoxyphenyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, pyrazyl, quinolyl, 3,5-dimethylisoxazolyl, 2-methylthiazolyl, thiazolyl, 2-thienyl, 3-thienyl and pyrimidyl; and

q is 0-2; and

n is 1 or 2.

2. A compound of formula (II)



wherein:

A, B, D, E, G and Q are as defined in claim 1;

K is H, (C5-C7) cycloalkyl, (C5-C6) aromatic ring, 1-[(C1-C4)-alkyl]-4-piperidinyl, 1-piperazinyl, 1-[(C1-C4)-alkyl]-4-piperazinyl, (C1-C7)-straight or branched alkyl, (C2-C7)-straight or branched alkenyl or alkynyl, wherein up to two carbon atoms in K are optionally replaced independently by O, S, SO, SO₂, NH, NO or N-(C1-C4)-alkyl,

wherein K optionally comprises up to 2 substituents independently selected from halo, amino, hydroxy, carboxy, methoxy or (C1-C3)alkyl; and

L and M are independently selected from H, (C1-C7)-straight or branched alkyl, (C2-C7)-straight or branched alkenyl or alkynyl, wherein one carbon atom in L and M is

optionally replaced by O, S, SO, SO₂, NH or N-(C1-C4)-alkyl,

wherein L and M optionally comprise up to two substituents independently selected from halogen, hydroxy, amino, carboxy, or a 5 to 6 membered aromatic ring, said aromatic ring comprising up to two heteroatoms selected from N, O or S; and n is 1 or 2.

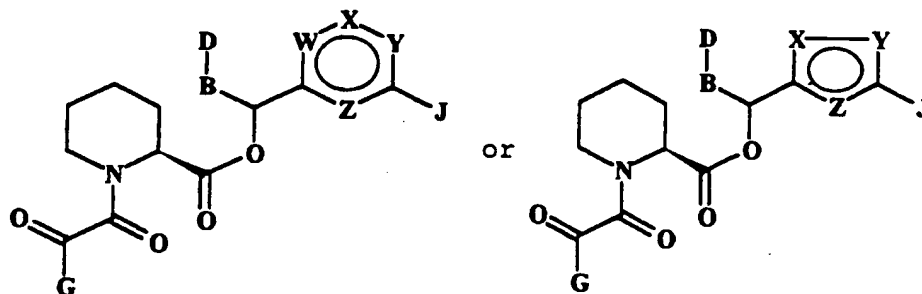
3. The compound according to claim 1 or 2, wherein no monocyclic or bicyclic ring in said compound comprises more than one heteroatom selected from O, S or N

4. The compound according to claim 1 or 2, wherein:

A is oxygen; and

E is -C(O)-C(O)-.

5. The compound according to claim 4, having the formulae:



wherein

B is propyl, ethyl, or 1-methylethenyl;

D is phenyl, N-morpholinyl, 4-hydroxy-cyclohexyl, 4-(N-methyl)-piperidinyl, 4-pyridyl or pyranyl;

G is 3,4,5-trimethoxyphenyl, 3,4-dimethoxyphenyl, 4-fluorophenyl, 2-furanyl, 1,1-dimethyl-2-methoxyethyl, t-butyl, 4-(4-hydroxy) pyranyl, isobutyl, 4-pyranyl, isobutyl, isopropyl, 1-methylcyclohexyl, 1,1,2-trimethylpropyl, 1-hydroxycyclohexyl, 1-trimethylpropyl, 4-

methoxy-1-hydroxy- cyclohexyl, 5-methoxymethyl-2-methylphenyl, 2-methylcyclohexyl, 5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl-2-enyl, 2-methylcyclohexyl, 5-(1-methyl-1-methoxyethyl)-2-methylcyclohexyl, 5-ethoxy-2-methylcyclohexyl, 4-ethoxy-N-aceto-2-pyrrolidinyl, or 5-isopropyl-2-methylcyclohexyl; and

J is 4-phenyl-1-(3-pyridyl)-1-butenyl, 2,5-diethoxyphenyl, 4-phenyl-1-(3-pyridyl-N-oxide)-1-butenyl, 2-methoxyphenyl, 1-(3-pyridyl)-1-pentenyl, 2-ethoxyphenyl, 2,5-dipropoxyphenyl, 2,6-dimethoxyphenyl, 1-(3-pyridyl)-1-butenyl, 1-(3-pyridyl)-1-pentenyl, 1-(3-pyridyl)-1-hexenyl, 1-(4-methylphenyl)-1-pentenyl, 2,6-dimethoxymethylphenyl, 1-cyclohexyl-1-pentenyl, 2-ethoxymethyl-N-indolyl, 1-cyclohexyl-3-methoxy-1-propenyl, 2,6-diethoxymethylphenyl, 1-(3-pyridyl)-1-hexa-1,5-dienyl, 1-(4-pyranyl)-1-hexa-1,5-dienyl, 1-cyclohexyl-1-hexenyl, 2,5-dipropyl-N-pyrrolyl, 2-methyl-5-butyl-N-pyrrolyl, 3-(1-methoxy)-2-hexenyl, 3-(1-methoxy)-4-methyl-2-pentenyl, 2,5-dimethyl-N-pyrrolyl, 3-(2-methyl)-3-heptenyl or 2-(2-hexenyl); and

W, X, Y and Z are independently selected from CH, N, O or S.

6. A pharmaceutical composition comprising an amount of a compound according to claim 1 to 2 effective for the treatment or prevention of multi-drug resistance and a pharmaceutically acceptable carrier, adjuvant or vehicle.

7. The pharmaceutical composition according to claim 6, further comprising a chemotherapeutic agent.

8. The pharmaceutical composition according to claim 7, wherein said chemotherapeutic agent is selected

from actinomycin D, doxorubicin, vincristine, vinblastine, etoposide, amsacrine, mitoxantrone, tenipaside, taxol or colchicine.

9. The pharmaceutical composition according to claim 6, further comprising a chemosensitizer.

10. The pharmaceutical composition according to claim 9, wherein said chemosensitizer is selected from cyclosporin A and its analogs, phenothiazines or thioxantheres.

11. A method for treating or preventing multi-drug resistance in a patient comprising the step of administering to said patient a pharmaceutical composition according to claim 6.

12. A pharmaceutically acceptable composition comprising:

- a) an amount of a compound according to claim 1 or 2 effective for stimulating neurite growth;
- b) a neurotrophic factor; and
- c) a pharmaceutically acceptable carrier, adjuvant or vehicle.

13. The composition according to claim 12, wherein said neurotrophic factor is selected from nerve growth factor (NGF), insulin growth factor (IGF) and active truncated derivatives thereof, acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), platelet-derived growth factors (PDGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factors (CNTF), glial cell line-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4/5).

14. A method for stimulating neurite growth in nerve cells comprising the step of contacting said nerve cells with a composition according to claim 12.

15. The method according to claim 14, comprising the additional step of contacting said nerve cells with a neurotrophic factor.

16. The method according to claim 15, wherein said neurotrophic factor is selected from nerve growth factor (NGF), insulin growth factor (IGF) and active truncated derivatives thereof, acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), platelet-derived growth factors (PDGF), brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factors (CNTF), glial cell line-derived neurotrophic factor (GDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT-4/5).

17. The method according to claim 14, wherein said method is used to treat a patient who is suffering from or has suffered from Alzheimer's disease, Parkinson's disease, ALS, stroke and ischemia associated with stroke, neural paropathy, other neural degenerative diseases, motor neuron diseases, sciatic crush, spinal cord injuries or facial nerve crush.